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EDITOR
HENRY CHANDLER COWLES

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ERRATA

Vol. XCV

P. 79, line 20, for "*rocki*" read "*rockii*"

P. 440, line 19, for "6 per cent NaOH" read "0.6 per cent NaOH"

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PRECIPITIN-RING TEST APPLIED TO FUNGI. II[1]

CONTRIBUTIONS FROM THE HULL BOTANICAL LABORATORY 445

GEORGE K. K. LINK AND HAZEL W. WILCOX

I. Review of literature

This paper is a report on investigations, begun in 1927, to determine whether the precipitin test is serviceable for identification and classification of certain phytopathogenic Ascomycetes and Fungi Imperfecti and some of their non-pathogenic taxonomic allies. Fractions extractable with 0.85% NaCl solution from hyphae and spores of these fungi were used to produce immune sera in rabbits and for preparation of test antigens.

Following the attempts of SCHÜTZE (15) to apply the precipitin test to identification and classification of yeasts used in the brewing industries, and of CITRON (1) to use it for identification and detection of fungi pathogenic to man, MAGNUS and FRIEDENTHAL (7) attempted to determine its adaptability for phylogenetic studies of fungi. In the course of extensive use of the precipitin test by the Königsberg school in phylogenetic studies of the entire plant kingdom, STEINECKE (14) applied the test to representatives of the algae and fungi, and NEUHOFF and ZIEGENSPECK (13) used it to study the relationships of Basidiomycetes and representatives of other classes

[1] This investigation was aided in part by a grant to the University of Chicago from the Rockefeller Foundation. Other acknowledgments have been made in a preceding paper (5).

of fungi. The conclusions drawn from these studies were summarized by MEZ (9, 10) and MEZ and ZIEGENSPECK (11). While the work of this school incited numerous investigations bearing upon the suitability of the precipitin test for phylogenetic and biochemical studies of higher plants, serological studies of the phytopathogenic fungi have received little attention. Literature pertaining to these studies of higher plants has been recently summarized by SILBERSCHMIDT (16) and hence need not be discussed here.

Recently several papers bearing on the application of the precipitin test to phytopathogenic fungi have appeared. MATSUMOTO (8) applied serological tests, among them the precipitin test, to *Aspergillus* species, and concluded that the method has possibilities. COONS and STRONG (2) attempted to apply the precipitin test to *Fusarium* species. They were unable to produce sera with sufficiently high titers for precipitin tests, and so abandoned it in favor of the complement-fixation test. NELSON (12) reported limited success with the precipitin test using purified globulins of *Fusarium lini*. Like all others who have used fungal thalli as antigen sources, he had difficulty in obtaining saline extracts sufficiently potent to give good titers with antisera. LIM and KUROTCHKIN (6), and KESTON et al. (3), working with fungi pathogenic to man, used as test antigens water-soluble polysaccharide fractions and reported very high titers.

II. Fungi used in test

To determine the efficacy of the precipitin test in differentiating the Fungi Imperfecti, representatives were chosen from the form genus *Fusarium*, which is taxonomically one of the most confusing genera of the Fungi Imperfecti, and phytopathogenically the most important division of the Phragmosporae. Identification and classification of the organisms considered species of this genus are often difficult or impossible, because when grown on artificial media they may fail to produce their distinguishing structures. Because of their biological and economic importance, the members of this genus seemed desirable material with which to begin a study of the antigenic and haptenic properties of phytopathogenic fungi.

In his latest contribution to the taxonomy of *Fusarium*, WOLLENWEBER (17) recognized 64 species with 79 varieties and 38 forms,

which he has arranged in 16 sections with 9 subsections, 12 of the sections including members for which the ascomycete stage has been established. This form genus is of additional interest because it includes many representatives which, although part of the etiological complex in diverse hosts or of distinct pathic events in the same host, are morphologically and physiologically differentiable only as varieties or forms.

The work was begun with three species of *Fusarium*, *F. conglutinans*, *F. cubense*, and *F. lycopersici*. It finally included the species and varieties listed in table I. The members of this list were added as the investigation progressed; consequently reciprocal tests have not been run with all of them. This list includes: (a) representatives which are known to be the conidial stages of different genera and species of the ascomycete order Hypocreales; (b) parasitic and nonparasitic representatives of the sections recognized by WOLLENWEBER (17); (c) pathogenic representatives which are characterized by host and even by symptom specificities, but which are not always readily differentiated morphologically.

The test was also applied to organisms so similar to *Fusarium* species that they had once been considered such. Hence *Cylindrocarpon album* (Sacc.) Wr. and a species of *Ramularia* were tested against one another and some of the *Fusarium* species. It was desirable to apply the test to members of the order Hypocreales which do not possess *Fusarium*-like conidial stages. Consequently *Neurospora tetrasperma* (Shear and B. O. Dodge) with monilioid conidia (*Monilia tetrasperma* Shear and Dodge) was used in tests with the conidial stage of *Gibberella saubinetii* (one way) and with other representatives with fusiform conidial fructifications.

Further experiments on relationship were made by testing members of the Hypocreales bearing fusiform or monilioid conidia, respectively, against members of the Pezizales with monilioid conidia or without macrospores, respectively. Consequently *Sclerotinia fructicola* (Wint.) Rehm, incitant of brown rot of stone fruits, which has a monilia stage, and *S. sclerotiorum* (Lib.) Mass., which bears no macroconidia but monilioid microconidia, were tested against the conidial stage of *N. tetrasperma* and several other representatives of the Hypocreales and of the genus *Fusarium*. Strains of *S. fructicola*

TABLE I

Name used in this paper	Name assigned by Wollenweber (17)	Incitant of	Section	Conidial stage of
F. acuminatum Ell. et Ev.	F. scirpi Lamb et Fautr. var. acuminatum (Ell. & Ev.) Wr.	Carnation wilt	X Gibbosum	....
F. anthophilum Wr.	F. arcuatum Berk. & Curt. var. majus Wr.	" "	VIII Roseum	....
F. argillaceum (Fr.) Sacc.	No change	....	XVI Ventricosum	Hypomyces solani Rke. et Berth.
F. cepae (Hanz) emend. Lk. and Bailey	F. oxysporum (Schl.) Wr.	Onion bulb rot	XIV Elegans; subsect. oxysporum	....
F. conglutinans Wr.	No change	Cabbage yellows	XIV Elegans; subsect. orthoceras	....
F. conglutinans Wr. var. callistephi Beach	No change	China aster wilt	XIV Elegans; subsect. orthoceras	....
F. cubense E.F.S.	F. oxysporum Schl. forma 3 Wr.	Banana wilt	XIV Elegans; subsect. oxysporum	....
F. decemcellulare Brick	No change	....	III Spicarioides	Calonectria rigidiuscula (Berk. et Brme.) Sacc.
F. dimerum Penzig	No change	....	I Eupionnotes	....
F. fructigenum Fr.	F. lateritium Nees var. fructigenum (Fr.) Wr.	Decay of stem and fruits, especially of trees	IX Lateritium	Gibb. baccatae (Wallr.) Sacc. var. evonymi (Fuckel) Wr.
F. graminearum Schwabe*	No change	Wheat seedling blight	XI Discolor; subsect. saubinetii	Gibb. saubinetii (Mont.) Sacc.
F. javanicum Koorders	No change	....	XV Martiella	....
F. lycopersici (Sacc.) Wr.	F. bulbigenum Cke et Mass. forma 1 Wr.	Tomato blight	XIV Elegans; subsect. constrictum	....
F. moniliforme Sheld.	No change	Corn rot	XIII Liseola	Gibb. moniliformis (Sheld.) Wineland
F. nivale (Fr.) Cesate	No change	Snow mold of cereals	VI Arachnites	Calonectria graminicola (Berk. et Brme.) Wr.

F. ossicolum (Berk et Curt) Sacc.	F. equiseti (Cda) Sacc. forma 1 Wr.	....	VI Gibbosum	....
F. oxysporum Schlechtendahl.	No change	Potato wilt	XIV Elegans; subsect. oxysporum	....
F. sambucinum Fuckel	No change	....	XI Discolor; subsect. saubinetii	Gibb. pulicaris (Fr.) Sacc.
F. semitectum Berk. et Rav.	No change	....	IX Arthrosporiella	....
F. sporotrichioides Sherb.	No change	Pea wilt	VII Sporotrichiella	....
F. theobromae App. et Strk.†	F. javanicum Koord. var. theobromae (App. et Strk.) Wr.	Cacao pod rot	XV Martiella	Hypomyces ipomoeae (Hals.) Wr.
F. trichothecioides Wr.	No change	Potato tuber dry rot	XI Discolor; subsect. trichothecioides	....

* Two cultures of *F. graminearum* were used. One, derived from an ascospore of *Gibb. saubinetii*, is designated as *Gibb. saubinetii #259*; the other, from an ascospore of an English strain of *Gibb. saubinetii*, is designated *Gibb. saubinetii* English strain.

† Two cultures of *F. theobromae* were used. One, derived from an ascospore of *Hypomyces ipomoeae*, is designated *H. ipomoeae;* the other, derived from a conidial culture of *F. theobromae*, is designated *F. theobromae*.

(two monoconidial cultures which had appeared sufficiently diverse in several years of culture to be considered separate entities and were identified by Dr. JOHN W. ROBERTS as *S. fructicola*) and *S. sclerotiorum* were tested against each other. Finally, plus and minus strains of *N. tetrasperma* were used for a study of genotypically distinct strains of the same species.

Sclerotinia sclerotiorum was cultured in its vegetative and sclerotial stage and is designated *S. sclerotiorum* in this paper. *S. fructicola* was cultured in the conidial monilia stage, the two strains being designated *S. fructicola* no. 1 and no. 2 respectively. *Neurospora tetrasperma* was cultured in the conidial stage, *Monilia tetrasperma* Shear and Dodge. The sexually distinct strains are designated *Neurospora tetrasperma* A and B respectively, and the mixed culture as *N. tetrasperma* A+B.[2]

III. Methods

A detailed report will not be given of every attempt made to devise a standard procedure, but only of those methods finally adopted.

CULTURE MEDIUM.—A search was made for a suitable protein-free liquid medium for *F. conglutinans*, *F. lycopersici*, and *F. cubense*. It was found that these organisms would grow in sufficient abundance in Richard's solution.[3] Cultures were grown in 100 cc. of solution in 250 cc. Erlenmeyer flasks because the yield per 1000 cc. of medium distributed in these units seemed to be greater than when larger units in larger flasks were used. Culture flasks were inoculated from a seeding flask (inoculated 4–5 days previously) containing a cloud of spores and hyphal fragments well dispersed by shaking. During the first days after inoculation the culture flasks were shaken to distribute the inoculum and then left undisturbed to facilitate mat production. Usually good mats developed in subdued light at 20°–

[2] All of these cultures were obtained from Dr. ALICE A. BAILEY except *Gibb. saubinetii*, obtained from Miss HELEN JOHANN; *F. nivale* obtained from Dr. A. S. DAHL; several strains of *F. cubense* obtained from Dr. J. R. JOHNSTON; *Cylindrocarpon album* and a *Ramularia* species obtained from Dr. C. D. SHERBAKOFF; *Neurospora tetrasperma* conidial strains A and B obtained from Dr. B. O. DODGE; and *Sclerotinia sclerotiorum* obtained from Dr. H. H. WHETZEL.

[3] 33.3 gm. sucrose, 6.6 gm. KNO_3, 3.3 gm. KH_2PO_4, and 1.7 gm. $MgSO_4$ in 1000 cc. distilled water.

30° C. within 3–4 weeks. All fungi used were grown in this medium. Some species grew more vigorously than others, so the yield of material (dry weight) per flask ranged from approximately 20 mg. to approximately 80 mg. If cultures were allowed to grow too long (2 months), the medium discolored, crystals formed which might become enmeshed in the mycelia, and black patches appeared on the lower side of the mats.

RECOVERY OF FUNGUS MATS.—Contents of the flask were poured upon filter paper (Schleicher and Schüll no. 595) on a Büchner funnel attached to a filter flask, and the medium drained off. The fungus mat was rapidly rinsed with three changes of distilled water and permitted to become semi-dry, at which time it could readily be peeled off the paper with forceps. Since extensive use was to be made of the fungus material for many months, it was necessary to grow large quantities and to dry the material preliminary to storage in tightly stoppered bottles. Various methods of drying with minimum opportunity for autolysis were tried. A drying apparatus was finally used which drew air at a temperature of 25°–30° C. over the damp fungus material loosely stacked in petri dishes. Mats were dried in 1–4 hours, depending on their thickness. The dried mats were stored in desiccators over sulphuric acid preliminary to pulverization.

PULVERIZATION OF MATS.—The mats were ground entirely in agate mortars, at first by hand fine enough to pass a 20-mesh sieve, then in an electrical grinding machine until sufficiently fine to pass a 100-mesh sieve. All attempts to grind the powder fine enough for suspensions usable in intravenous injections failed. The particles always swelled sufficiently to bring about the death of the animal. Powder prepared as just described when used in suspensions was suitable for intraperitoneal injection, such a suspension readily passing a 20-gauge needle.

EXTRACTION OF POWDERS.—The aim was to obtain clear extracts with sufficient protein content to make tests possible, yet free from lipoids which might interfere with the clarity of the test antigen and specificity of the precipitin test.

Preliminary trials on lipoid extraction involved using alcohol alone, in various concentrations, or using alcohol preceding or following ether extraction in the cold or in the reflux condenser. Petrol

ether (ligroin) (B.P. range 30°–60° C. max. B.P. 80°) was found to be the best lipoid solvent for these organisms. To minimize possible chemical change and loss of proteins, no extractions of the powders with alcohol and/or ether were made at temperatures above 25° C. For this same reason treatment of the material with selenium oxychloride as used by COONS and STRONG (2) was not adopted.

The following method of lipoid extraction was employed. Petrol ether was added to the desired amount of powder, shaken occasionally, and allowed to act on it for 1–15 hours at 25° C. After centrifugation this ether was decanted and fresh ether added. Usually three or four changes sufficed to give a fat-free test when a drop of ether was evaporated on a watch crystal. The ether was then decanted and the powder thoroughly dried preliminary to saline extraction. Using this method, powders extracted with 0.85% NaCl solution gave biuret tests, whereas some of the non-lipoid extracted powders did not. It was also found that in some cases (*F. cubense*, *F. lycopersici*, *F. conglutinans*) this treatment tended to enhance the antigenic properties of the injected material and the reactive properties of the test antigens. However, many of the fungus powders (*F. javanicum*, *F. fructigenum*) gave clear test antigens without lipoid extractions.

Grinding of fresh or dry fungus mats by hand or in a ball mill with various neutral or alkaline extractives was tried. This was abandoned because antigens sufficiently clear for intravenous injection or use as test antigens were not obtainable from the ground material, despite filtration and centrifugation. When only this material was injected intraperitoneally, it did not produce adequate titers for tests.

As suggested by MEZ (9), fungus powders were ground with pulverized pumice. A weighed amount of the powder was ground by hand with an equal volume of ether-extracted pulverized pumice preliminary to extraction with petrol ether and with salt solution. Since in some test antigens this procedure slightly increased the potency of the reaction, it was incorporated into the standard procedure.

Saline extractions of both inject and test antigen were tried for various periods of time at different temperatures to ascertain optimum conditions.

Following is a synopsis of the standard preparation of the antigens. For the test antigen, 1:50 stock dilution, 0.3 gm. of powder was ground with pumice (3 minutes), extracted with petrol ether until fat-free, dried, and extracted in 15 cc. of 0.85% saline solution for 18 hours at 0° C. After centrifugation, sparkling clear extracts were obtained. A few organisms used, such as the *Sclerotinia* species, required a longer centrifugation at higher speed. Extracts of several powders in concentrations greater than 1:50 (1:10 or 1:20) were readily made by this method. To prepare a clear 1:20 extract of *S. sclerotiorum*, however, filtration through hard filter paper (Whatman's no. 50) caked with fuller's earth (Hyflo) was required after centrifuging. For the injection antigens, powders were extracted directly in saline without preliminary pumice and petrol ether treatment. All antigens were stored at 0° C. Fresh lots of test antigens were usually prepared every 2 weeks. When stored a month or more they became slightly more acid but gave apparently valid reactions.

IMMUNIZATION.—Attempts to produce titers by intravenous injection of either clear extracts or suspended fungal material failed, the former because the titers were too low; the latter because the animals quickly succumbed to embolism. Neither did intraperitoneal injections alone prove adequate to incite potent antisera. A combination of intravenous injection of a clear saline extract of the powder and of intraperitoneal injection of the extracted powder resuspended in saline was found to give adequate titers.

The following standard procedure was used. For each series of injections per animal, 0.3 gm. of powder was extracted in 10 cc. of saline at 0° C. for approximately 16 hours, then separated by centrifugation to obtain a clear supernatant liquid. This liquid (8 cc. approximately) was used for intravenous injection; the residue, resuspended in 10 cc. of saline, was used for intraperitoneal injections (12 cc. approximately). Animals were bled for normal serum and then injected on three consecutive days according to the following schedule:

	INTRAVENOUS INJECTION	INTRAPERITONEAL INJECTION
1st day	2 cc.	3 cc.
2nd day	3 cc.	4 cc.
3rd day	Remainder (3 cc.+)	Remainder (4 cc.+)

After a 5-day interval of rest this procedure was repeated. When the desired number of series of injections had been given, the animal was taken off feed the fourth day and bled the fifth day after the last injection was received. Two such series of injections usually produced antisera with adequate titers, some as high as 1:25,600; but in most cases approximately 1:3200. (An antiserum produced by this schedule is designated I_2 throughout this paper.) Forty cc. of blood furnished sufficient serum for tests against 20 to 25 antigens, allowing an adequate reserve for supplementary tests. No preservatives were added to the antisera, which were obtained aseptically and stored at 0° C. with relatively slight loss by spoilage.

Hot weather, moulting, feeding animals shortly before bleeding, all seemed to be factors which might contribute to opalescent serum. Such correlations were not consistent, however; the antisera of some animals never appeared opalescent, while those of others were frequently so.

PRECIPITIN TEST.—Numerous preliminary trials were made determining the most effective use of antisera and of test antigens to obtain maximum titers.

Tests were run with graded antigen dilutions against a constant serum (undiluted and diluted 1:1) and with graded serum dilutions against a constant antigen dilution. A 0.85% NaCl solution was used exclusively for the antigen dilutions; 10% glucose, glycerin, 1.7 and 2.55% NaCl solutions were used in serum dilutions. Tests with glucose-diluted serum gave sharp interphases but slightly lower titers; glycerin-diluted serum showed very sharp interphases but the titers were considerably diminished. Serum diluted with hypertonic saline showed very slight, if any, lowering in intensity of reaction. Serially diluted serum run against a constant antigen dilution could not be used because the density of the higher dilutions was such that clear interphases were not obtained.

Since sufficient serum could be obtained from each animal for tests with 20–24 test antigens, it was used undiluted against serial dilutions of antigens. Test antigen dilutions were prepared with 0.85% NaCl solution from a 1:50 stock extract of each fungus powder, to yield graded dilutions of 1:100, 1:200, 1:400, 1:800, to 1:6400, and when necessary, up to 1:25,600.

Approximately 0.05 cc. of each serum was distributed with a capillary pipette into each tube of a series of pyrex precipitin tubes (5 mm. diameter). The serum in each tube was then layered with approximately 0.05 cc. of the appropriate antigen dilution, separate pipettes being used for each dilution. The tubes were incubated at room temperature (28°–32° C.) and read at 1 and 2-hour intervals after layering. The symbols ±, 1, 2, 3, and 4 were used to represent the intensity of reaction as measured by the thickness and density of the layer of precipitate (ring) formed at the interphase; ± being the faintest discernible line, 4 the broadest, densest ring obtained, the other symbols representing intermediate values.

Preliminary studies showed that rings may appear at any time from less than one minute to 30 minutes after contact of antiserum and test antigen. Most of the second hour readings varied only slightly from those of the first hour, the usual difference consisting of a slight additional thickening of the rings to the next higher value (that is, a 2 ring became a 3 ring), or in the appearance of the last detectable reaction (±) in the next higher dilution (that is, a titer of 1:3200 of the first hour reading might rise to 1:6400 by the close of the second hour). Third and fourth hour readings usually were unsuccessful because extensive diffusion of the two fluids rendered the rings indefinite and their evaluation inaccurate. Attempts to estimate total precipitate by readings made after shaking the tubes (following the second hour reading) and holding them at 0° C. overnight proved unsatisfactory because of the difficulty in estimating the small amount of precipitate present, owing to the extreme curvature of the bottom of the tube.

Although extensive absorption tests were run, no one technique has yet been found which will give consistent results with all sera or with different samples of the same serum. The absorbing agents were the powders added directly to the sera, or extracts of powders, 1:10 in the *Fusarium* species and 1:20 in the *Sclerotinia* species studied. Powders were not extracted with petrol ether except in preparation of the extracts of the *Sclerotinia* species. Most rapid and complete absorption was obtained by using two volumes of extract to one volume of serum, mixing the two fluids thoroughly, and then incubating the tubes in a water bath (40° C.). After a suitable period

of incubation the tubes were transferred to 0° C., stored over-night, and centrifuged until clear the following day. Tests were run immediately with the decanted supernatant liquid against the standard series of test antigen dilutions. When required, test antigen dilutions under 1:50 were prepared from the extract used as the absorbing agent.

When powders were used occasional shaking was necessary to keep the powder in suspension. (Sera were more likely to be opalescent when absorbed with powder than when absorbed with extracts.) Although the time of incubation at 40° and amount of powder used varied with the history of the serum and particular organism used, approximately 30 mg. of powder per cc. of serum (or 2 volumes of extract to 1 volume of serum), incubated 4–8 hours, then refrigerated, fell within the range of partial or complete absorption. With this trial as a working basis, variations could be made to obtain desired results. Absorption schedules using refrigeration before and after incubation at 40° showed no consistent differences in results when compared with those of tests run as described.

For control tests, the serum of the normal animal was tested with the same antigens (lower dilutions) against which the immune serum would be run, and against the saline used in preparing these antigens. At least two animals were immunized per organism, and in preliminary experiments each serum was run in duplicate as an additional control. The latter was found unnecessary as different readings of the same serum varied scarcely at all.

IV. Results

Data here recorded were obtained by the standard procedure outlined in the preceding section. No attempt will be made to present the full protocols of all experiments. All but one of the animals immunized (*F. sporotrichioides*) produced antisera with sufficiently high titers for tests after two series of immunizations. Normal sera of a few animals (13) gave feeble reactions with one or two antigens (notably *F. oxysporum*, *F. dimerum*, and *Gibb. saubinetii*) in the lower dilutions (up to 1:200); normal serum of only one animal reacted with many antigens at 1:200 or above, and consequently this serum was not used. The protocols of tests with normal sera are not given since all that were later used as antisera gave negative reac-

tions in the normal tests. Full reciprocal tests were not made between all organisms because all antigens and antisera were not available simultaneously.

The data of tables II, III, IV, and VI are selected and presented on the following bases:

1. Reactions of I_2 sera (produced by *two* standard series of injections).

2. Antiserum with highest titer.

3. Second hour readings (highest titer in duplicate series of tests chosen, when run).

4. The heading Titer represents the highest dilution of the given test antigen with which the given antiserum reacted, including $\pm$ reactions.

5. The heading Sum represents the total of reactions of a serum in all dilutions from 1:100 on, $\pm$, 1, 2, 3, and 4 being arbitrarily assigned the numerical values 5, 10, 20, 30, and 40 respectively. Intermediate reactions were assigned intermediate values; for example, $3- = 25$.

6. The Percentage heading represents the relative percentage value of the sums of the reactions of a given serum with each test antigen, the homologous reaction being taken at 100%.

7. Homologous values for each serum and antigen are printed in heavy type.

Table II gives the protocols for three pairs of animals immunized with *F. argillaceum*, *F. fructigenum*, and *F. ossicolum* respectively. It shows: (1) typical protocols of tests with antisera (first hour readings are omitted); (2) differences in sera of animals immunized concurrently; (3) the derivation of data of tables III, IV, and VI from full protocols (Titer, Sum, and % appear in their respective columns to the right of each serum).

Tables III and IV record such data synoptically for all organisms investigated, protocols being selected by the criteria previously given. Additional columns at the end of these tables record: (1) the number of antisera per organism from which the cited antiserum was selected; (2) the number of stock test antigen lots prepared per organism; (3) the pH range of these antigen lots (1:100 dilution tested); (4) the most frequent pH of the antigen lots.

TABLE II

Organism	F. argillaceum antisera (I_2)																		
	Antiserum #86*								Antiserum #87*								Titer	Sum	%
	1:50	1:100	1:200	1:400	1:800	1:1600	1:3200	1:6400	1:50	1:100	1:200	1:400	1:800	1:1600	1:3200	1:6400			
F. *anthophilum*	±	—	—	—	—	—	—	—	±	—	—	—	—	—	—	—	50	....	....
F. *argillaceum*	3	3	3	2	1	±	±	—	4	4	4	4	3	2	1	±	6400	185	100
F. cepae	±	—	—	—	—	—	—	—	1	1 —	±	±	—	—	—	—	400	15	8
F. conglutinans	—	—	—	—	—	—	—	—	±	±	±	—	—	—	—	—	200	10	5
F. conglutinans var. callestephi	±	±	—	—	—	—	—	—	±	—	—	—	—	—	—	—	50	....	....
F. cubense #8	—	—	—	—	—	—	—	—	±	±	±	—	—	—	—	—	200	10	5
F. *decemcellulare*	±	±	—	—	—	—	—	—	±	±	—	—	—	—	—	—	100	5	2
F. dimerum	—	—	—	—	—	—	—	—	1	±	—	—	—	—	—	—	100	5	2
F. fructigenum	±	±	—	—	—	—	—	—	±	±	—	—	—	—	—	—	100	5	2
F. javanicum	±	—	—	—	—	—	—	—	1	1 —	±	—	—	—	—	—	200	10	5
F. lycopersici	—	—	—	—	—	—	—	—	±	±	±	—	—	—	—	—	200	10	5
F. *moniliforme*	—	—	—	—	—	—	—	—	±	±	±	±	—	—	—	—	400	15	8
F. *nivale*	—	—	—	—	—	—	—	—	±	±	±	±	±	—	—	—	800	20	10
F. ossicolum	—	—	—	—	—	—	—	—	1	±	±	±	±	—	—	—	800	20	10
F. semitectum	—	—	—	—	—	—	—	—	1	±	±	—	—	—	—	—	200	10	3
F. *sporotrichioides*	—	—	—	—	—	—	—	—	±	±	±	±	±	—	—	—	400	20	31
F. trichothecioides	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	0	0	0
Gibberella saubinetii #259*	....	....	....	....	....	....	....	....	....	....	....	....	....	....	....	....	....	....	....
F. acuminatum	....	....	....	....	....	....	....	....	....	....	....	....	....	....	....	....	....	....	....

* Saline controls with all sera negative.

TABLE II—*Continued*

Organism	F. fructigenum antisera (I_2)																				
	Antiserum #80*									Antiserum #81*									Titer	Sum	%
	1:50	1:100	1:200	1:400	1:800	1:1600	1:3200	1:6400	1:12800	1:50	1:100	1:200	1:400	1:800	1:1600	1:3200	1:6400	1:12800			
F. anthophilum	2	2	2	1	—	—	—	—	—	3	3	2	1	±	—	—	—	—	800	65	28
F. argillaceum	—	—	—	—	—	—	—	—	—	±	—	—	—	—	—	—	—	—	50	....	13
F. cepae	2	2	1	1	—	—	—	—	—	3	2	1	—	—	—	—	—	—	200	30	
F. conglutinans	2	2	2	1	—	—	—	—	—	3	3	2	1	—	—	—	—	—	400	60	26
F. conglutinans var. callestephi	2	2	2	1	±	—	—	—	—	3	3	2	1	1	±	—	—	—	1600	75	33
F. cubense #8	2	2	2	1	—	—	—	—	—	2	2	2	1	±	—	—	—	—	800	55	24
F. decemcellulare	±	±	—	—	—	—	—	—	—	1	±	—	—	—	—	—	—	—	100	5	2
F. dimerum	2	2	1	1	±	—	—	—	—	4—	3—	2	1	±	—	—	—	—	800	60	26
F. fructigenum	4	4	4	4	3	2	1	±	—	4	4	4	4	4	3	2	1	±	**12,800**	**225**	**100**
F. javanicum	2	2	2	2	1	±	—	—	—	3	3	3	3	2	2—	1	±	—	6400	140	61
F. lycopersici	4	4	3	2	1	±	±	—	—	3	3	3	2	1	1	—	—	—	1600	100	44
F. moniliforme	2	2	1	—	—	—	—	—	—	3—	2	1	±	—	—	—	—	—	400	35	15
F. nivale	—	—	—	—	—	—	—	—	—	2	2	1	±	—	—	—	—	—	400	35	15
F. ossicolum	±	±	±	—	—	—	—	—	—	1	1	1	1	±	—	—	—	—	800	35	15
F. semitectum	1	±	—	—	—	—	—	—	—	1	1	1	1	—	—	—	—	—	400	30	13
F. sporotrichioides	1	1	±	—	—	—	—	—	—	2	2	2	1	±	—	—	—	—	800	55	24
F. trichothecioides	—	—	—	—	—	—	—	—	—	2	2	1	1	1	±	—	—	—	1600	55	24
Gibberella saubinetii #259*	1	±	—	—	—	—	—	—	—	3	2	1	±	—	—	—	—	—	400	35	15
F. acuminatum	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....

TABLE II—*Continued*

Organism	F. ossicolum antisera (I_2)																			
	Antiserum #82*								Antiserum #83*									Titer	Sum	%
	1:50	1:100	1:200	1:400	1:800	1:1600	1:3200	1:6400	1:50	1:100	1:200	1:400	1:800	1:1600	1:3200	1:6400	1:12800			
F. anthophilum	3	3	3	3	1	±	—	—	4	4	4	4	2	1	±	—	—	3200	155	164
F. argillaceum	2—	±	—	—	—	—	—	—	±	—	—	—	—	—	—	—	....	50	....	....
F. cepae	3	2	1	±	—	—	—	—	3	2	2—	±	±	—	—	—	....	800	45	47
F. conglutinans	4	3	2	1	—	—	—	—	4	3	2	1	±	—	—	—	....	800	65	68
F. conglutinans var. callestephi	2	3	2	1	±	—	—	—	3	4	3	2	1	±	—	—	....	1600	105	11
F. *cubense* #8	3	3	2	1	±	—	—	—	3	3	3	2	1—	—	—	—	....	800	85	90
F. decemcellulare	3	2	1	±	±	—	—	—	2	2	±	—	—	—	—	—	....	200	25	26
F. dimerum	2	2	2	1	±	—	—	—	2	2	2	1	±	—	—	—	....	800	55	58
F. fructigenum	4	4	4	4	3	2	1	±	4	4	4	4	4	3	2	1	±	12,800	225	238
F. javanicum	4	4	4	4	3	2—	±	—	4	4	4	4	3	1	±	—	....	3200	165	174
F. lycopersici	4	4	4	2	1	1—	—	—	4	4	4	3—	2	1	±	—	....	3200	140	148
F. *moniliforme*	3	2	1	±	—	—	—	—	3—	2—	2—	1	±	—	—	—	....	800	45	47
F. nivale	2	2—	1	—	—	—	—	—	3	2	1	±	—	—	—	—	....	400	35	37
F. ossicolum	4	4	3	2	1	±	—	—	4	4	4	1	±	—	—	—	....	**800**	**95**	**100**
F. semitectum	2	2	1	1	—	—	—	—	4—	3	3—	2	1—	—	—	—	....	800	80	84
F. sporotrichioides	3	3	2	2	1	1	—	—	3	3	3	2	1	±	—	—	....	1600	95	100
F. trichothecioides	4	4	3	1	±	—	—	—	4	4	3	2	1	±	—	—	....	1600	105	111
Gibberella saubinetii #259*	3	2	1	±	—	—	—	—	3	3—	3—	1	—	—	—	—	....	400	60	63
F. acuminatum	4	4	3	2	±	—	—	—	4	4	3	1	±	—	—	—	....	......	....	....

TABLE III

IMMUNE SERA

	Organism	1			2			3			4*			5			6			7		
		TITER	SUM	%	TITER	SUM	%	TITER	SUM	%	TITER	SUM	%	TITER	SUM	%	TITER	SUM	%	TITER	SUM	%
1	F. anthophilum	**3200**	**145**	**100**	50	....	....	400	30	30	1600	60	51	400	20	10	1600	130	72	200	20	50
2	F. argillaceum	200	20	13	**6400**	**185**	**100**	200	10	10	800	25	21	0	0	0	200	20	10	0	0	0
3	F. cepae	400	25	17	400	15	8	**1600**	**100**	**100**	1600	45	39	400	50	27	1600	105	56	0	0	0
4	F. conglutinans	400	40	27	200	10	5	800	55	55	**6400**	**115**	**100**	3200	100	55	3200	155	83	1600	75	187
5	F. conglutinans var. callistephi	1600	70	48	50	....	....	800	100	100	6400	115	100	**12,800**	**180**	**100**	6400	205	110	1600	75	187
6	F. cubense #8	800	55	37	200	10	5	800	45	45	1600	65	55	3200	105	56	**3200**	**180**	**100**	1600	75	187
7	F. decemcellulare	100	5	3	100	5	2	100	5	5	800	40	34	50	....	....	200	20	10	**800**	**40**	**100**
8	F. dimerum	400	30	20	100	5	2	400	25	25	3200	55	47	100	10	5	800	80	43	400	40	100
9	F. fructigenum	3200	110	75	100	5	2	800	50	50	3200	100	86	6400	130	60	19,200	175	97	3200	105	262
10	F. javanicum	1600	60	41	200	10	5	800	35	35	6400	110	95	100	5	2	12,800	205	110	3200	100	250
11	F. lycopersici	3200	90	62	200	10	5	800	80	80	3200	70	60	3200	155	85	3200	180	100	1600	85	212
12	F. moniliforme	400	35	24	200	15	8	800	45	45	1600	55	47	800	65	35	1600	110	59	400	25	62
13	F. nivale	50	....	....	800	20	10	200	15	15	800	35	29	50	....	....	400	50	27	100	5	12
14	F. ossicolum	200	10	6	800	20	10	800	25	25	3200	65	55	50	....	....	1600	105	56	100	5	12
15	F. oxysporum	....	....	....	....	....	....	....	....	....	....	....	....	....	....	....	....	....	....	....	....	....
16	F. semitectum	100	5	3	200	10	5	200	15	15	800	40	34	50	....	....	400	35	18	50	....	....
17	F. sporotrichioides	800	45	31	400	20	10	800	35	35	1600	55	47	200	10	5	1600	120	64	400	30	75
18	F. theobromae	....	....	....	....	....	....	....	....	....	....	....	....	....	....	....	....	....	....	....	....	....
19	F. trichothecioides	200	15	10	0	0	0	800	30	30	1600	80	68	50	....	....	3200	105	56	200	20	50
20	Gibberella saubinetii #259	200	15	10	100	5	2	800	20	20	1600	35	29	200	20	10	6400	95	51	400	45	112
21	Hypomyces ipomoeae	....	....	....	....	....	....	....	....	....	....	....	....	....	....	....	....	....	....	....	....	....
22	Cylindrocarpon album	....	....	....	....	....	....	....	....	....	....	....	....	....	....	....	....	....	....	....	....	....
23	Ramularia sp.	....	....	....	....	....	....	....	....	....	....	....	....	....	....	....	....	....	....	....	....	....
24	Neurospora tetrasperma A+B	....	....	....	....	....	....	....	....	....	....	....	....	....	....	....	....	....	....	....	....	....
25	Sclerotinia sclerotiorum	....	....	....	....	....	....	....	....	....	....	....	....	....	....	....	....	....	....	....	....	....
26	F. acuminatum	....	....	....	....	....	....	....	....	....	....	....	....	....	....	....	....	....	....	....	....	....
27	F. sambucinum	....	....	....	....	....	....	800	50	50	....	....	....	....	....	....	....	....	....	....	....	....
28	Gibberella saubinetii (English strain)	....	....	....	....	....	....	....	....	....	....	....	....	....	....	....	....	....	....	....	....	....

* Sum represents total of reactions in all dilutions from 1:200 on.

TABLE III—*Continued*

Organism	8			9			10			11			12			13			14		
	Titer	Sum	%	Titer	Sum	%	Titer	Sum	%	Titer	Sum	%	Titer	Sum	%	Titer	Sum	%	Titer	Sum	%
1 F. anthophilum	100	15	14	800	65	28	800	55	31	1600	55	39	400	25	33	800	65	61	3200	155	164
2 F. argillaceum	0	0	....	0	0	0	400	35	20	100	5	3	200	15	19	100	10	9	50	....	....
3 F. cepae	200	20	19	200	30	13	200	20	11	1600	105	75	400	35	46	400	25	23	800	45	47
4 F. conglutinans	1600	55	52	400	60	26	200	10	5	800	65	46	3200	100	132	50	....	....	800	65	68
5 F. conglutinans var. callistephi	1600	75	72	1600	75	33	400	40	23	3200	180	129	3200	110	145	800	30	28	1600	105	111
6 F. cubense #8	800	70	67	800	55	24	400	30	17	3200	150	108	3200	85	112	50	....	....	800	85	90
7 F. decemcellulare	100	5	4	100	5	2	200	15	8	100	5	3	0	0	0	200	15	14	200	25	26
8 F. dimerum	**1600**	**105**	**100**	800	60	26	400	20	11	200	20	14	800	25	33	200	30	28	800	55	58
9 F. fructigenum	3200	115	110	**12,800**	**225**	**100**	6400	155	89	6400	145	104	6400	120	158	800	65	61	12,800	225	238
10 F. javanicum	1600	75	72	6400	140	61	**3200**	**170**	**100**	3200	85	61	1600	75	100	800	40	38	3200	165	174
11 F. lycopersici	1600	105	100	1600	100	44	3200	115	66	**3200**	**140**	**100**	3200	115	151	400	25	23	3200	140	148
12 F. moniliforme	400	40	38	400	35	15	200	15	8	800	65	46	**1600**	**75**	**100**	400	35	33	800	45	47
13 F. nivale	200	20	19	400	35	15	200	10	5	50	....	....	200	20	26	**1600**	**105**	**100**	400	35	37
14 F. ossicolum	400	25	24	800	35	15	200	20	11	100	20	14	400	20	26	400	35	33	**800**	**95**	**100**
15 F. oxysporum	....	....	....	....	....	....	200	15	....	....	....	....	....	....	....	....	....	....	....	....	....
16 F. semitectum	200	15	14	400	30	13	200	25	14	50	....	....	200	10	13	800	80	75	800	80	84
17 F. sporotrichioides	400	65	62	800	55	24	400	20	11	800	45	32	400	30	39	400	15	14	1600	95	100
18 F. theobromae	....	....	....	....	....	....	1600	65	37	....	....	....	....	....	....	....	....	....	....	....	....
19 F. trichothecioides	400	30	28	1600	55	24	400	25	14	400	30	21	200	10	13	200	15	14	1600	105	111
20 Gibberella saubinetii #259	800	45	43	400	35	15	800	20	11	400	35	25	1600	80	105	400	35	33	400	60	63
21 Hypomyces ipomoeae	....	....	....	....	....	....	3200	155	89	....	....	....	....	....	....	....	....	....	....	....	....
22 Cylindrocarpon album	....	....	....	....	....	....	400	30	17	....	....	....	....	....	....	....	....	....	....	....	....
23 Ramularia sp.	....	....	....	....	....	....	50	....	....	....	....	....	....	....	....	....	....	....	....	....	....
24 Neurospora tetrasperma A+B	....	....	....	....	....	....	0	0	0	....	....	....	....	....	....	....	....	....	....	....	....
25 Sclerotinia sclerotiorum	....	....	....	....	....	....	0	0	0	....	....	....	....	....	....	....	....	....	....	....	....
26 F. acuminatum	....	....	....	....	....	....	....	....	....	....	....	....	....	....	....	....	....	....	800	85	90
27 F. sambucinum	....	....	....	....	....	....	....	....	....	....	....	....	....	....	....	....	....	....	....	....	....
28 Gibberella saubinetii (English strain)	....	....	....	....	....	....	....	....	....	....	....	....	....	....	....	....	....	....	....	....	....

TABLE III—*Continued*

Organism	15			16			17			18			19			20			21		
	TITER	SUM	%	TITER	SUM	%	TITER	SUM	%	TITER	SUM	%	TITER	SUM	%	TITER	SUM	%	TITER	SUM	%
1 F. anthophilum	100	5	7	200	15	23	1600	130	78	1600	100	74	400	50	50	200	15	14	1600	40	26
2 F. argillaceum	0	0	0	50	....	....	800	55	33	200	15	11	0	0	0	100	5	4	100	5	3
3 F. cepae	200	35	49	200	25	38	1600	140	84	400	25	18	200	15	15	200	15	14	1600	35	23
4 F. conglutinans	50	....	....	200	15	23	3200	175	106	200	20	14	800	65	65	400	35	32	0	0	0
5 F. conglutinans var. callistephi	400	15	21	800	70	107	6400	205	124	400	50	37	3200	125	125	400	35	32	1600	35	23
6 F. cubense #8	0	0	0	200	35	53	6400	185	109	800	55	40	1600	95	95	400	35	32	1600	50	33
7 F. decemcellulare	50	....	....	50	....	....	800	100	60	400	30	22	200	15	15	100	10	9	200	20	13
8 F. dimerum	200	15	21	800	100	154	3200	145	87	800	45	33	1600	105	105	400	35	32	400	25	16
9 F. fructigenum	200	15	21	6400	185	284	12,800	215	130	6400	175	129	3200	130	130	800	60	56	1600	105	69
10 F. javanicum	200	10	14	3200	165	254	6400	145	87	3200	175	129	1600	110	110	800	45	42	1600	140	92
11 F. lycopersici	1600	40	56	3200	145	223	3200	190	115	1600	130	96	3200	145	145	1600	105	100	3200	95	62
12 F. moniliforme	400	35	49	400	45	69	3200	150	90	400	35	25	800	90	90	400	35	32	400	20	13
13 F. nivale	400	25	35	400	55	84	1600	120	72	200	15	11	400	60	60	800	45	42	800	30	19
14 F. ossicolum	400	10	14	800	80	123	6400	170	103	800	40	29	800	100	100	400	25	23	1600	45	29
15 F. oxysporum	**800**	**70**	**100**	....	....	....	....	....	....	800	30	22	....	....	....	....	....	....	6400	60	39
16 F. semitectum	200	15	21	**800**	**65**	**100**	3200	15	90	1600	55	40	800	45	45	200	20	18	3200	60	39
17 F. sporotrichioides	400	30	42	800	65	100	**3200**	**165**	**100**	400	40	29	400	60	60	400	45	42	3200	70	46
18 F. theobromae	....	....	....	....	....	....	....	....	....	**1600**	**135**	**100**	....	....	....	....	....	....	400	45	....
19 F. trichothecioides	100	5	7	400	45	69	3200	135	87	400	60	44	**800**	**100**	**100**	800	45	42	1600	55	36
20 Gibberella saubinetii #259	0	0	0	800	70	107	1600	80	48	800	40	29	800	80	80	**1600**	**105**	**100**	800	25	16
21 Hypomyces ipomoeae	0	0	0	....	....	....	....	....	....	3200	180	133	....	....	....	....	....	....	**3200**	**150**	**130**
22 Cylindrocarpon album	0	0	0	....	....	....	....	....	....	1600	55	40	....	....	....	....	....	....	6400	55	36
23 Ramularia sp.	50	....	....	....	....	....	....	....	....	800	55	40	....	....	....	....	....	....	200	10	6
24 Neurospora tetrasperma A+B	0	0	0	....	....	....	....	....	....	50	....	....	....	....	....	....	....	....	0	0	0
25 Sclerotinia sclerotiorum	100	10	14	....	....	....	....	....	....	50	....	....	....	....	....	....	....	....	400	15	10
26 F. acuminatum	....	....	....	....	....	....	....	....	....	....	....	....	....	....	....	....	....	....	....	....	....
27 F. sambucinum	....	....	....	....	....	....	....	....	....	....	....	....	800	45	45	....	....	....	....	....	....
28 Gibberella saubinetii (English strain)	....	....	....	....	....	....	....	....	....	....	....	....	....	....	....	800	65	61	....	....	....

TABLE III—*Continued*

Organism	22			23			24			25			No. immune sera per organism	No. stock antigen lots prepared	pH range of stock antigen lots	Most frequent pH of stock antigen lots
	Titer	Sum	%	Titer	Sum	%	Titer	Sum	%	Titer	Sum	%				
1 F. anthophilum	400	15	33	0	0	0	400	20	23	0	0	0	2	16	4.9-6.0	5.4
2 F. argillaceum	0	0	0	0	0	0	0	0	0	0	0	0	3	25	5.4-6.4	6.1
3 F. cepae	0	0	0	100	5	16	800	50	59	0	0	0	3	18	5.1-6.1	5.9
4 F. conglutinans	0	0	0	0	0	0	1600	35	41	0	0	0	8	21	6.0-6.6	6.2
5 F. conglutinans var. callistephi	0	0	0	0	0	0	3200	75	88	0	0	0	8	27	5.7-6.4	5.9
6 F. cubense #8	50	....	....	0	0	0	3200	60	70	0	0	0	5	27	6.6-7.1	6.8
7 F. decemcellulare	0	0	0	0	0	0	400	20	23	0	0	0	2	17	5.7-6.1	5.9
8 F. dimerum	100	5	11	100	10	33	3200	45	53	100	5	2	2	17	5.3-6.1	5.8
9 F. fructigenum	50	....	....	50	....	....	400	20	23	0	0	0	2	29	6.5-7.0	6.7
10 F. javanicum	100	5	11	200	10	33	1600	55	64	50	....	....	9	33	5.6-6.5	6.1
11 F. lycopersici	200	20	44	0	0	0	800	60	70	0	0	0	6	28	5.5-6.0	5.7
12 F. moniliforme	0	0	0	200	10	33	1600	45	53	0	0	0	2	26	5.1-5.6	5.3
13 F. nivale	0	0	0	0	0	0	800	30	35	0	0	0	3	18	5.1-5.7	5.3
14 F. ossicolum	0	0	0	0	0	0	1600	50	59	0	0	0	2	17	5.1-5.6	5.3
15 F. oxysporum	400	25	55	200	15	49	400	30	35	400	20	14	2	12	5.6-5.7	5.6
16 F. semitectum	0	0	0	0	0	0	200	10	11	0	0	0	2	18	5.3-5.9	5.6
17 F. sporotrichioides	0	0	0	0	0	0	800	40	47	0	0	0	5	17	4.9-5.6	5.1
18 F. theobromae	....	....	....	....	....	....	....	....	....	....	....	....	4	7	5.5-6.0	5.9
19 F. trichothecioides	0	0	0	0	0	0	800	40	47	0	0	0	5	20	5.6-6.7	5.8
20 Gibberella saubinetii #259	0	0	0	0	0	0	800	35	41	0	0	0	4	18	5.1-5.8	5.4
21 Hypomyces ipomoeae	0	0	0	0	0	0	3200	55	64	0	0	0	8	18	5.6-6.1	5.9
22 Cylindrocarpon album	**800**	**45**	**100**	200	15	49	1600	45	53	800	30	22	2	2	5.5-6.6	....
23 Ramularia sp.	50	....	....	**200**	**30**	**100**	400	25	29	50	....	....	2	2	5.7-5.8	....
24 Neurospora tetrasperma A+B	0	0	0	0	0	0	**1600**	**85**	**100**	100	5	3	2	5	5.6-6.2	6.1
25 Sclerotinia sclerotiorum	100	10	22	100	5	16	100	15	18	**6400**	**135**	**100**	6	21	5.0-6.2	....
26 F. acuminatum	....	....	....	....	....	....	....	....	....	....	....	....	....	2	5.7-6.0	....
27 F. sambucinum	....	....	....	....	....	....	....	....	....	....	....	....	....	2	5.7-6.0	....
28 Gibberella saubinetii (English strain)	....	....	....	....	....	....	....	....	....	....	....	....	....	3	5.7-5.8	....

TABLE IV

IMMUNE SERUM

ORGANISM	6A			31			32			24			33			34			25A			No. IM-MUNE SERA PER ORGAN-ISM	No. STOCK ANTI-GEN LOTS PRE-PARED	pH RANGE OF STOCK ANTI-GEN LOTS	MOST FRE-QUENT pH OF STOCK ANTI-GEN LOTS
	TITER	SUM	%	TI-TER	SUM	%	TI-TER	SUM	%	TI-TER	SUM	%	TI-TER	SUM	%	TI-TER	SUM	%	TI-TER	SUM	%				
29 F. cubense #4	12,800	240	111	....	....	....	....	....	....	....	....	....	....	....	....	....	....	....	....	....	....	......	2	......	......
30 F. cubense #5	6,400	160	74	....	....	....	....	....	....	....	....	....	....	....	....	....	....	....	....	....	....	......	2	......	......
6a F. cubense #8	**12,800**	**215**	**100**	....	....	....	....	....	....	....	....	....	....	....	....	....	....	....	....	....	....	5	27	6.6-7.1	6.8
31 Neurospora tetrasperma A	....	....	....	**1600**	**75**	**100**	100	5	16	1600	90	105	....	....	....	....	....	....	....	....	....	5	10	5.5-6.0	6.0
32 N. tetrasperma B	....	....	....	800	55	72	**200**	**30**	**100**	1600	105	123	....	....	....	....	....	....	....	....	....	5	10	5.6-6.9	5.7
24 N. tetrasperma A+B	....	....	....	1600	85	112	200	10	33	**1600**	**85**	**100**	....	....	....	....	....	....	....	....	....	2	5	5.6-6.2	6.1
33 S. fructicola #1	....	....	....	....	....	....	....	....	....	....	....	....	**800**	**40**	**100**	100	10	22	1600	50	35	2	6	5.6-5.9	5.7
34 S. fructicola #2	....	....	....	....	....	....	....	....	....	....	....	....	800	35	87	**1600**	**45**	**100**	400	25	17	3	6	5.9-6.2	6.0
25a S. sclerotiorum	....	....	....	....	....	....	....	....	....	....	....	....	3200	65	162	1600	60	133	**3200**	**140**	**100**	6	21	5.0-6.2	......

Protocols of table II represent three of the types of reaction found upon analysis of the serological data. Tables III and IV show additional instances, including limited tests, of these and other types, which are summarized as follows:

1. Antigen and serum specific. . . F. argillaceum, F. oxysporum, S. sclerotiorum
2. Antigen and serum specific except in 2–3 cases. . . . . F. fructigenum, F. anthophilum, F. nivale, F. cepae, F. conglutinans, F. dimerum, F. conglutinans var. callistephi, Gibb. saubinetii no. 259, Hypomyces ipomoeae, Neurospora tetrasperma A+B, N. tetrasperma B, Ramularia sp., S. fructicola no. 1, S. fructicola no .2
3. Antigen specific; serum nonspecific. F. theobromae, F. sporotrichioides
4. Antigen non-specific; serum specific. F. javanicum, Cylindrocarpon album
5. Antigen and serum nonspecific. F. ossicolum, F. trichothecioides

The remaining unlisted organisms fall into a group of intermediate specificity for both serum and antigen. The most nonspecific serum was that of *F. decemcellulare*, which reacted higher with 8 out of 20 heterologous antigens than with its homologous antigen. The most non-specific antigen was that of *F. lycopersici*, which reacted higher with 7 out of 24 heterologous antisera than with its homologous antiserum. The sera against which most test antigens gave non-specific reactions were *F. cubense* no. 8 and *F. sporotrichioides*. The test antigens against which most sera gave non-specific reactions were *F. fructigenum*, *F. lycopersici*, *F. conglutinans* var. *callistephi*, and *F. javanicum*. Several other antigens and sera gave non-specific reactions, but only in one or two cases each.

The specific precipitability of saline extracts of the *Sclerotinia*

species on the one hand, and of the *Fusarium*, *Cylindrocarpon*, *Ramularia*, and *Neurospora* species on the other, was sufficiently marked to permit differentiation of members of the Pezizales from members of the Hypocreales. Among members of the Hypocreales this specificity was sufficient to permit ready differentiation between the groups mentioned. Differentiation between members of the *Fusarium* group and *Neurospora* was less marked than that between the *Fusarium* group, *Cylindrocarpon*, and *Ramularia*.

Although the *Sclerotinia* species gave marked cross reactions, differences exist in the precipitabilities of the extracts of *S. sclerotiorum* and *S. fructicola*. Neither the plus and minus strains of *N. tetrasperma* nor the two strains of *S. fructicola* could be separated. Similarly the extracts of the *Fusarium* species gave such marked cross reactions (excepting *F. argillaceum*) that sharp differentiation between them was not possible.

Hence the test did not readily differentiate all entities which are separable by morphological and physiological criteria such as host and symptom specificity. However, although many of the organisms studied were not separable by the precipitin test, a consideration of all reactions of every organism leads to the conclusion that each organism is a distinct serological entity.

V. Supplementary tests and results

These experiments, undertaken to investigate failure of the precipitin test to differentiate fungi separable by morphological and physiological criteria, consisted of: (1) Tests with antisera to determine the effect of additional immunizing series after the I_2 series; decline of titer *in vivo* and *in vitro;* effect of long rest periods and subsequent reimmunizations. (2) Study of the pH of immune sera and of test antigen lots; extraction and dilution of the latter in buffered saline solutions of different pH values to find the effect of different hydrogen-ion concentrations upon the reaction between a given test antigen and serum. (3) Determination of the total nitrogen content of powders and extracts by micro-Kjeldahl tests (4) and study of the effect of equivalent and different nitrogen contents of inject and test antigens upon the titer of given antisera. (4) Precipitin absorption tests.

1. ANTISERA.—It was found, in general, that if the titers for homologous and heterologous test antigens were high in an antiserum prepared by two series of injections (I_2), a third series of injections (I_3) would raise both titers slightly. If, however, the homologous reaction was high and the heterologous reaction low, the titer of the latter was raised more, proportionately, than that of the former after the animal had received a third series of injections. Additional series in either type bring about a gradual rise in titer of both homologous and heterologous reactions. This rise is sometimes erratic for a given antigen against different lots of the same serum. Thus an antigen may give a lower reaction against an antiserum tested after five series of injections than it did against the same serum tested after four series of injections.

Incidental observations were made relative to the titer of antisera stored in and out of the animal body. In general, when several weeks had elapsed after the final series of injections, the titer fell more rapidly *in vivo* than *in vitro*. For example, an antiserum of *F. fructigenum* prepared with three series of injections and stored *in vitro* for six weeks gave a higher titer against heterologous antigens than did serum taken from the animal four weeks after immunization. Homologous reactions in the two were the same. In another sample of this serum kept *in vivo* six weeks and then tested, the heterologous reactions were almost gone and the homologous titer was decidedly lower than in the two samples just cited.

The greater the number of immunization series preceding an interval of rest, the less drastic is the drop in titer during that period. Antiserum of an animal immunized with two series of injections may still show approximately half of its titer after 18 weeks of storage *in vitro*. Many sera, however, showed substantial diminution in titer after 4 or 5 weeks of storage. After periods of 10 or 12 weeks tests might reveal the following trends in titer change: (1) nearly all reactions ceasing or reduced to $\pm$ in the dilution 1:200; (2) strong homologous and heterologous reactions disappearing while weak heterologous reactions remained apparently unchanged; (3) a homologous reaction persisting longer than an equally high heterologous reaction.

Record was made also of the effect of additional series of injections

received a long interval after the two initial series. An antiserum prepared by two such series after a 16-week interval showed a strong advance in titer for all antigens, much greater than that exhibited after the two initial series of injections. Injection with a third series produced no great change from that achieved by the two preceding series, heterologous reactions still showing a proportionately higher rise than homologous reactions. After 16 weeks' storage following reimmunization, this serum showed less drop in titer than it had in the same interval following the two initial series of injections.

The conclusion was drawn that the number and intervals of injection series used for standard immunization were not responsible for the non-specific reactions noted.

2. EFFECT OF HYDROGEN-ION CONCENTRATION ON SPECIFICITY OF TESTS.—Experiments were made to determine whether the hydrogen-ion concentration of antisera and test antigens was a factor in the non-specific reactions obtained. All readings were made electrometrically on the 1:100 dilution of test antigens and on undiluted serum. As an additional check some readings were made colorimetrically on the same material.

Since the pH of the antisera tested ranged from 7.3 to 7.8, 7.5–7.6 being the most frequent value, it was concluded that the hydrogen-ion concentration of antisera was not a critical factor. The pH of the stock saline used in preparation of antigens ranged from 5.5 to 6.7, approximately 5.8 being the most frequent value.

Tables III and IV show that considerable variations occurred in the pH of different test antigens, and in the pH of different lots of the same test antigen. They also show that although all such antigens were acid, none was extremely so. The tables indicate that there is no consistent correlation between the potencies of test antigens and their hydrogen-ion concentration. Thus, although the most frequent pH of test antigen lots of *F. conglutinans* var. *callistephi* and *F. decemcellulare* is the same, the former antigen has a much stronger reaction against all sera than has the latter.

To check this preliminary conclusion, detailed studies were made with nine species of *Fusarium* and with *Sclerotinia sclerotiorum*. The control lots were prepared according to the standard procedure, with unbuffered saline of approximately pH 6.0. Comparable lots of test

antigen were prepared using 0.85% NaCl solution buffered to give pH values of 4.4, 5.0, 6.0, 7.0, and 7.6. Modifications of test antigen solutions were of three types: (1) powder extracted with saline buffered to pH 4.4 and dilutions made with 4.4 saline; (2) powder extracted with unbuffered saline (6.0) and dilutions made with 4.4 saline; (3) powder extracted with saline buffered to 4.4 and dilutions made with unbuffered NaCl. This series was repeated using pH values 5.0, 6.0, 7.0, and 7.6.

The first type of extraction and dilution was applied to all nine organisms, the second to many, and the third to only a few. Homologous tests were run with each antigen, and in most cases, tests with several heterologous sera, so that in all, nine test antigens, each with 6 to 16 modifications, were run against several members of a group of 18 antisera.

Titers obtained with buffered preparations of test antigens showed little variation from those obtained with unbuffered preparations of stock antigens (standard procedure). Greatest deviation was noted in tests with antigens buffered to pH 4.4 and 5.0. These titers were usually lower than those of the other buffered lots. Atypical behavior was especially noticeable at pH 4.4, such as: (1) reaction occurring when there was none in the other antigen preparations; (2) very weak reactions extending without gradation to the limit of the dilutions tested; (3) "rings" in the control tube (not with all sera).

Powders extracted and diluted with 4.4 and 5.0 buffer lots were slightly less potent than those extracted with unbuffered saline (pH 6.0) and diluted with saline buffered to pH 4.4 and 5.0.

Since these anomalous reactions occurred in a pH range below that of test antigens prepared by the standard method, it is concluded that the variations in titer and non-specific reactions observed were not materially affected by the pH of the saline used in preparation of the stock antigens.

3. MICRO-KJELDAHL TESTS.—On the assumption that quantitative differences in the nitrogen content of the extracts and powders might be a factor in the non-specific reactions noted, a series of total nitrogen determinations was made using the modified KOCH and McMEEKIN (4) micro-Kjeldahl test. Several representative fungus

powders were analyzed. Samples of the following were used: saline extracts prepared from powder extracted with petrol ether; saline extracts prepared from untreated powders; powder extracted with petrol ether; untreated powders. The results are given in table V.

TABLE V

Organism	Milligrams of nitrogen in			
	1 cc. 1:50 saline extract of powder	1 cc. 1:50 saline extract of ligroin-treated powder	10 mg. of untreated powder	10 mg. of ligroin-treated powder
F. anthophilum	....	....	0.35	....
F. argillaceum	0.27	....	....	....
F. cepae	....	....	0.48	....
F. conglutinans	0.15	....	0.45	....
F. conglutinans var. callistephi	0.10–0.20*	....	0.51	....
F. cubense #8	0.10–0.22	....	0.45	....
F. decemcellulare	0.15	....	0.33	....
F. dimerum	....	....	0.39	....
F. fructigenum	0.15–0.53	0.49	0.51–0.54	0.27
F. javanicum	0.34	0.35	0.49	0.49
F. lycopersici	0.20	....	0.50	....
F. oxysporum	0.59	....	0.61	....
F. theobromae	0.33	0.43	0.39	0.55
Gibb. saubinetii #259	0.14	....	....	....
Gibb. saubinetii (English)	0.21	....	....	....
Hypomyces ipomoeae	0.41	0.44	0.46	0.49
Cylindrocarpon album	0.25	....	0.40	....
Ramularia sp.	0.22	....	0.29	....
Neurospora tetrasperma A+B	0.46	....	0.44	....
S. sclerotiorum	0.10	0.08	0.15	0.11

* Two numbers indicate range where difference in tests is great.

The nitrogen content of the powders and saline extracts varied considerably. A comparison of tables III, IV, and V, however, does not show a significant correlation between the potency of a given antigen or antiserum and the nitrogen content of that organism's powder or extract. Thus, test antigens of *F. decemcellulare* which produced relatively low titers with all antisera contained as much nitrogen as those of *F. conglutinans* which produced relatively high titers with these antisera.

To check this observation, experiments were run with test and inject antigens of closely related organisms standardized against an arbitrary unit to contain the same quantity of nitrogen. The cultures selected for the test are designated *Hypomyces ipomoeae* and *F. theobromae*, and supposedly are two cultures of the same taxonomic entity, *F. javanicum* var. *theobromae*, which is supposed to be the conidial stage of *Hypomyces ipomoeae*. The culture designated *F. javanicum* supposedly is the imperfect species of which the culture of *F. theobromae* is a variety.

Antisera were prepared of *Hypomyces ipomoeae*, *F. theobromae*, and *F. javanicum*. Test antigens were prepared for each and for *S. sclerotiorum* and *F. fructigenum*. Control antisera for each organism were produced by two series of injections, each series containing 0.3 gm. of powder (standard immunization). Another set of antisera were prepared per organism with two series of injections, each series containing amounts of powder equivalent in nitrogen to a given unit. Similarly, standardized test antigen lots were prepared. For example, using the nitrogen content of 0.3 gm. of powder of *Hypomyces ipomoeae* as a standard, 0.36 gm. of powder of *F. javanicum* had to be used per set of injections to supply an equivalent amount of nitrogen. (As a reciprocal control, a pair of animals received 0.245 gm. of powder of *H. ipomoeae* per series of injections. This amount was approximately equal in nitrogen content to 0.3 gm. of powder of *F. javanicum* or *F. theobromae*.) Similarly, using the 1:50 stock extract of *H. ipomoeae* (0.3 gm. of powder in 15 cc. saline) as a standard, the nitrogen equivalent extract of *F. javanicum* was made from 0.37 gm. of powder in 15 cc. of saline. Results of this experiment are given in table VI.

The chemical analysis and serological tests do not reveal identity of the organisms as one would expect. In fact, the culture designated *F. javanicum* so far as nitrogen content and serological reaction are concerned stands closer to the culture designated *H. ipomoeae* than the culture designated *F. theobromae*.

It was found that the antisera of animals receiving inject antigens supposedly containing the same amounts of nitrogen showed differences in titer equal to those observed in animals immunized with the standard amounts of powders. Similarly, when test antigens sup-

posedly containing equivalent amounts of nitrogen were used in comparative tests with standard test antigens, the same magnitude and orders of difference in titer were noted in both.

The conclusion was drawn that quantitative difference in nitrogen content of inject and test antigens is not a critical factor in explaining the non-specific results obtained.

TABLE VI

IMMUNE SERA I_2

Organism	Hypomyces ipomoeae				F. theobromae				F. javanicum			
	0.3 gm.*		0.245 gm.*		0.3 gm.*		0.37 gm.*		0.3 gm.*		0.36 gm.*	
	Titer	Sum	Titer	Sum	Titer	Sum	Titer	Sum	Titer	Sum	Titer	Sum
Hypomyces ipomoeae (0.3)†	6400	175	3200	115	3200	185	6400	185	6400	155	3200	145
Hypomyces ipomoeae (0.3)†	6400	170	3200	140	6400	185	6400	180	6400	175	3200	135
F. theobromae (0.3)†	1600	65	800	60	1600	140	1600	105	1600	75	1600	75
F. theobromae (0.307)	1600	60	800	60	3200	100	1600	105	1600	70	1600	70
F. javanicum (0.3)†	3200	160	1600	105	6400	180	3200	145	6400	135	1600	95
F. javanicum (0.37)	6400	145	1600	90	6400	160	3200	130	3200	140	1600	105
F. fructigenum (0.3)†	3200	95	1600	85	6400	145	1600	120	3200	125	800	55
F. fructigenum (0.27)	1600	70	1600	70	6400	175	1600	120	3200	115	800	60
S. sclerotiorum (0.3)†	0	0	0	0	0	0	0	0	0	0	0	0
S. sclerotiorum (0.825)‡	0	0	0	0	0	0	0	0	0	0	0	0

* This figure represents number of grams of powder received by each animal per series of injections.

† This figure represents grams of powder used in 1:50 stock test antigen dilution.

‡ This figure represents grams of powder used in 1:50 stock test antigen dilution, one-half the N equivalent amount.

4. PRECIPITIN ABSORPTION TESTS.—This series of experiments was undertaken in an attempt to determine whether or not absorption of precipitins would afford differentiation of organisms where the precipitin test failed to do so.

Fragmentary tests were run with *F. cepae* vs. *F. oxysporum*, *F. fructigenum* vs. *F. callistephi*, *F. javanicum* vs. *Hypomyces ipomoeae*, *F. nivale* vs. *F. argillaceum*, and *Gibb. saubinetii* no. 259 vs. *Gibb. saubinetii* (English strain). Fuller tests were run with *F. conglutinans* var. *callistephi*, *F. cubense* no. 8, and *F. lycopersici*, reciprocally. Detailed intensive tests were run with *F. conglutinans* vs. *F. lycopersici*,

Neurospora tetrasperma (single ascospore strain A) vs. *N. tetrasperma* (single ascospore strain B), (two lots of antigen), and *S. sclerotiorum* vs. two strains of *S. fructicola*.

Although in the section on methods some suggestions as to approximate amounts of extract or powder and serum, and periods and types of incubation were given, a routine method of precipitin absorption has not yet been found. The suggestions indicate a possible range of partial or complete absorption within which the desired conditions might be attained.

For every serum-antigen combination used there was absorption. In some cases this was complete, the absorbed serum giving no reaction against the antigen with which it had been absorbed; in others absorption was partial, as indicated by the diminished titer of the serum and appropriate antigen; or serum was overabsorbed, as indicated by anomalous reactions. There were a few absorption tests among the many run in which proper absorption occurred. In the detailed studies on the *Sclerotinia* species, results obtained from partial absorption checked those obtained in complete absorption. In the detailed studies on the *Neurospora* strains, partial absorption results checked complete absorption results with very few exceptions. (For a second lot of powder grown in each case, these absorptions were all incomplete and therefore inconclusive.) Detailed studies on *F. conglutinans* and *F. lycopersici* showed that partial absorptions checked complete absorption except that all absorption results with serum obtained shortly after immunization were of one type while all of those with serum obtained some weeks after immunization were of a different type.

The absorptions obtained did indicate the possession by each organism of a specific antigenic fraction as well as a common antirenic fraction shared with the other organism. Examples are *N. tetraspermum* A vs. *N. tetraspermum* B, *S. sclerotiorum* vs. *S. fructicola* nos. 1 and 2, and *S. fructicola* no. 1 vs. *S. fructicola* no. 2, data for which are given in tables VII–IX.

On the whole, results obtained with absorption of precipitins were inconclusive because of the unreliability of the technique used. Thus a method giving complete absorption at one time might fail to do so when the tests were repeated using a sample of the same serum and

different antigen lots, or the same antigen and different serum lots. Nevertheless the data indicate that the precipitin absorption meth-

TABLE VII

Organism	Unabsorbed sera			Absorbed sera								
	F_1	F_2	S	F_1/F_1†	F_1/F_2	F_1/S	F_2/F_1	F_2/F_2	F_2/S	S/F_1	S/F_2	S/S
S. fructicola #1 (F_1)...	400*	800	200	0 C‡	100 P	200 S	0 C	0 C	200 N	0 C	0 C	0 C
S. fructicola #2 (F_2)...	400	400	200	50 P	40 P	400 S	40 P	0 C	200 S	40 P	0 C	100 P
S. sclerotiorum (S)....	800	800	3200	0 C	0 C	0 C	0 C	0 C	0 C	800 S	800 S	200 P

TABLE VIII

Organism	Unabsorbed sera		Absorbed sera			
	F_1	F_2	F_1/F_1†	F_1/F_2	F_2/F_2	F_2/F_1
S. fructicola #1 (F_1).......	200*	200	0 C‡	40 P	0 C	0 C
S. fructicola #2 (F_2).......	100	200	0 C	40 ±?	0 0	50 P

TABLE IX

Organism	Unabsorbed sera		Absorbed sera			
	A	B	A/A	A/B	B/B	B/A
N. tetrasperma A (A).....	1600*	800	0 C‡	30 P	0 C	0 C
N. tetrasperma B (B).....	1600	1600	0 C	0 C	0 C	30 P

* Numerals represent titers.

† In F_1/F_1, etc., numerator represents serum; denominator, absorbing antigen.

‡ C, complete absorption; P, partial absorption; S, very slight absorption; N, no absorption of homologous precipitin by heterologous organism.

od has possibilities, and when adequately controlled it may be an alternative means of differentiation in cases in which the precipitin test fails.

These supplementary tests indicate that variations due to age and history of antisera, variable hydrogen-ion concentration of test antigens, and variable nitrogen contents of powders and extracts are not individual limiting factors which might account for the non-specific reactions obtained. The results with absorption of precipitins indicate that the presence of common antigenic and haptenic substances may be responsible for some of the non-specific precipitations. Perfection of this technique gives promise of sharper differentiations.

Further possibilities that might account for the non-specific reactions obtained are: (1) the specific fractions of the organism may not be extractable with 0.85% NaCl solution (such as certain polysaccharides and lipoids); (2) the extract may be too diverse chemically. Extracts of greater chemical homogeneity, such as the purified globulins prepared from cell contents by NELSON (12), and from the different constituents of the hyphal walls, may make differentiation possible. In the present study specific reactions may have been masked in part by reactions of common cell wall constituents; (3) absorption of precipitins on spores and hyphal fragments may be more serviceable than such absorptions with extracts and powders.

VI. Summary

1. Potent antisera and test antigens (giving maximum titers of 1:25,600) were prepared from 34 species and strains of fungi by using fractions soluble in 0.85% NaCl solution.

2. The fractions of some organisms exhibited sufficient specificity to permit differentiation, but in most cases cross precipitin reactions were so strong that identification was impossible. In a few cases absorption of precipitins differentiated fungi not separable by the precipitin test.

3. Thus in a limited number of reciprocal tests, members of the Pezizales (*Sclerotinia* species) were differentiated from members of the Hypocreales (*Neurospora tetrasperma*, *Fusarium* species, and a species each of *Cylindrocarpon* and *Ramularia*), certain ones of which were in turn distinguished from one another. Strong group reactions occurred among all members of the genus *Fusarium* tested. The precipitin test did not permit as sharp differentiation between the

monilioid strains of *N. tetrasperma* and the *Fusarium* group as it did between *Fusarium* species and the conidial stages of *Cylindrocarpon album* and a *Ramularia* sp.

4. Two genera of the Pezizales (*S. sclerotiorum* and *S. fructicola*) and two strains of *S. fructicola*, as well as the plus and minus strains of *N. tetrasperma*, were differentiated by means of the precipitin absorption test.

5. Many attempts to demonstrate specific precipitabilities for the saline extracts of certain cultures recognized as strains and species of *Fusarium* (some of which are recognized as conidial stages of different genera of Ascomycetes) were unsuccessful.

6. At present, therefore, the procedure described in this paper is not invariably usable for identification of every one of the fungi tested, although consideration of all reactions of every organism leads to the conclusion that each is a distinct serological entity.

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EFFECTS OF TEMPERATURE ON METABOLISM IN TOMATO[1]

G. T. NIGHTINGALE

(WITH TWO FIGURES)

Introduction

In the practice of agriculture, it has always been recognized that seasonal climatic changes exert a dominant influence on plant growth, and for years this was considered to be due mainly to temperature. In 1920, GARNER and ALLARD (10) demonstrated that plant response was definitely affected by the seasonal length of day or photoperiod. It has also been shown that photoperiodic influences or effects may be materially modified by nutrient treatment (18). Obviously, plants are affected by all factors of the environment to which they are subjected. It is the purpose of this paper to point out certain effects of temperature.

Experimental methods

Plants of the tomato (*Lycopersicon esculentum* Mill.) were employed for these experiments, the Bonny Best variety being selected. The plants were propagated from seed and grown in 4-inch clay pots in sifted loam soil until they had reached a height of about 8 cm. At that time the roots were washed free of foreign material and set

[1] Through the courtesy of the University of Chicago there was most generously made available for these experiments the temperature-humidity control equipment and laboratory facilities of its Department of Botany. Some of the results thus obtained are reported in the following pages. Other papers will appear subsequently in this journal; and in the Contributions from Boyce Thompson Institute will be published the results of the work of ECKERSON (9), who made microchemical analyses, anatomical observations, and reducase determinations on the plants used for these experiments.

Kjeldahl and mineral determinations were made in the laboratory of C. S. CATHCART, for whose cooperation the writer wishes to express appreciation. He is also indebted to G. B. ULVIN, who assisted in making macro-determinations of chlorophyll. Special acknowledgment is due C. H. HARRISON, who for the two months preceding the actual temperature experiments grew the plants in sand culture, giving daily attention to nutrient applications and producing plants of the quality of growth desired for the experiments.

in washed quartz sand in new, washed, 10-inch clay pots, five plants to a pot. The pots were then set in shallow enamelware pans, and until the time of temperature treatments all of the plants received daily applications of minus-N nutrient solution (table I) in sufficient quantity to flush the sand and fill the pans. If the solution in the pans became low between periods of nutrient application, distilled water was added, although this was not often necessary. During the period preceding the time when they were subjected to the experimental temperature treatments, the plants were grown under usual greenhouse conditions with a temperature of 70°–75° F. during the day and of 65°–70° F. during the night.

TABLE I

COMPOSITION OF NUTRIENT SOLUTION (PARTIAL VOLUME MOLECULAR CONCENTRATIONS OF SALTS USED)

	$Ca(NO_3)_2$	KH_2PO_4	$MgSO_4$	$CaCl_2$
Complete or plus-NO_3......	0.0090	0.0045	0.0045	....
Minus-N................	....	0.0045	0.0045	0.0090

On April 26, 1932, after about six weeks of minus-N treatment, some of the plants (hereafter referred to as *initial* plants) were harvested for chemical analysis and the remainder were placed in the glass inclosed chambers designed for control of temperature and humidity. The glass of these chambers and of the greenhouse roof materially reduced light intensity as compared with that in the open. Air in the chambers was changed continuously and rapidly. It was obtained from outside the greenhouse and contained no artificially added carbon dioxide. These factors in relation to the results obtained will be discussed elsewhere. Three chambers were employed, with a relative humidity of 85 per cent in all of them, but the temperatures were 55°, 70°, and 95° F. respectively (13°, 21°, and 35° C.). Sudden changes in intensity of sunlight resulted in temporary fluctuations in temperature. In any case, however, the temperature did not vary more than ±1.5° from the standard set, and then for only a few minutes at any given time. Humidity was also controlled with about the same degree of accuracy.

Each temperature chamber accommodated 20 pots, or 100 plants. For a period of 19 hours all the plants continued to receive the minus-N nutrient treatment at the respective temperatures indicated. At the end of that time half the plants at each temperature were given the complete or plus-NO_3 nutrient solution (table I), and the remainder continued to receive the solution lacking nitrogen. These nutrient solutions were in all cases thereafter applied daily in the manner already described.

The plants were divided for analysis into fibrous roots, whole stems, blades, and petioles, the last portion including also the rachis and large veins. Only the results of macro-analyses of stems are reported in detail, however; the other parts of the plant followed very closely in trend the changes which occurred in the stem.

Determinations of nitrogenous and carbohydrate fractions were made with fresh and dried tissues respectively, according to procedure previously described in detail (**20, 21**). Aliquots of dried tissue were employed for mineral analyses (**1**).

Chlorophyll was extracted from representative aliquots of fresh blades according to the method of SCHERTZ (**31**) and the amount estimated colorimetrically using GUTHRIE'S (**11**) reagent as a standard.

Results

INITIAL PLANTS

The initial plants, as well as those used for experimental treatments, were selected from a large population and were uniform in size, quality, and appearance. On April 26, after six weeks of minus-N treatment, they were, as might be anticipated, typical nitrogen-deficient plants (fig. 1). The stems were about 25 cm. long but stiff and woody. The base of the stem was purplish blue to light yellow. The upper two or three leaves were fairly dark green but the lower ones were distinctly yellow, with purple veins. There were practically no blossoms present. The root system was unusually large in proportion to the tops and consisted of many fine white extensive roots.

The plants were high in carbohydrates but very low in all forms of organic nitrogen, and contained neither nitrate nor ammonium (tables II–V). Cell walls of mechanical and conductive tissue were

FIG. 1.—Experiments started on April 26, 1931: On left, typical *initial* tomato plants representative of those employed for experimental treatments. The three sets of plants on right are as they appeared on May 2, after receiving nutrient solution lacking nitrogen and the temperature treatments indicated (plants at 95° F. were considerably taller and more spindling at the time of final harvest on May 5).

thick, and starch was observed in large quantities in all parenchymatous tissue nearly to the tip of the stem (9).

Macro-analyses for minerals which are not reported in detail showed that the initial plants contained an abundance of phosphorus, potassium, calcium, magnesium, and sulphur.

ECKERSON (9) found that the leaves, especially of the initial plants, were very high in nitrate-reducing material. The plants were in every respect in ideal condition for protein synthesis and rapid growth, if given an available nitrate supply and favorable conditions of environment.

RESPONSES OF PLANTS AT 70° F.

1. PLANTS DEFICIENT IN NITRATES.—These plants were harvested for analysis on May 5, ten days after being subjected to a continuous temperature of 70° F. During that period there was comparatively little change in the appearance (fig. 1) and chemical composition (tables II–VI) of the plants as compared with their initial condition on April 26. There was, however, a slight increase in volume. They appeared darker green and there was increase in chlorophyll (table VI). A similar response but in greater degree was exhibited by the minus-N 95° plants.

2. PLANTS WHICH RECEIVED THE COMPLETE NUTRIENT SOLUTION.—Nitrate absorption was apparently instantaneous (9) and nitrate was found in all parts of the plant about four hours following application of the complete nutrient solution. Absorption of nitrate was accompanied by rapid reduction to nitrite, ammonium, and amino acid synthesis (9). Thirty-six hours after the plants received nitrate the leaves were visibly darker green. When finally harvested for analysis on May 5, the plants had increased greatly in volume (fig. 2), the new stem growth and new leaves were soft and succulent, and all parts of the plant were dark green and growing vigorously. No blossoms formed on this nor on any other group of plants during the ten-day period of temperature treatments.

Accompanying vigorous growth there was a marked decrease in concentration of carbohydrates (tables II, III) and increase in organic nitrogen (tables IV, V), and with change from yellowish to

Fig. 2.—Experiments started on April 26, 1931: On left, typical *initial* tomato plants representative of those employed for experimental treatments. The three sets of plants on right are as they appeared on May 2, after receiving complete nutrient solution and the temperature treatments indicated (at time of final harvest on May 5, the plants at 70° F. had increased at least a third more in volume and those subjected to 95° had increased in height, but the new growth was extremely soft and spindling).

dark green the percentage chlorophyll content of the blades more than doubled (table VI).

TABLE II

CARBOHYDRATE FRACTIONS OF WHOLE STEMS EXPRESSED AS PERCENTAGE OF DRY MATTER, AND DRY MATTER EXPRESSED AS PERCENTAGE OF GREEN MATTER

	PERCENTAGE						
	APRIL 26	MAY 5			MAY 5		
	INITIAL PLANTS	MINUS-N			PLUS-NO_3		
		55° F.	70° F.	95° F.	55° F.	70° F.	95° F.
Dry matter......	14.00	14.60	13.60	12.60	12.20	8.60	10.00
Reducing sugars.	11.57	11.57	8.60	11.15	7.29	6.80	7.15
Sucrose.........	3.42	8.98	7.53	3.58	4.85	2.47	2.74
Total sugars.....	14.99	20.55	16.13	14.73	12.14	9.27	9.89
Starch and dextrin	17.33	22.15	24.05	7.87	16.60	9.88	2.55
Total carbohydrates........	32.32	42.70	40.18	22.60	28.74	19.15	12.44

TABLE III

CARBOHYDRATE FRACTIONS OF WHOLE STEMS EXPRESSED AS PERCENTAGE OF GREEN MATTER

	PERCENTAGE						
	APRIL 26	MAY 5			MAY 5		
	INITIAL PLANTS	MINUS-N			PLUS-NO_3		
		55° F.	70° F.	95° F.	55° F.	70° F.	95° F.
Reducing sugars.	1.62	1.69	1.17	1.40	0.89	0.58	0.72
Sucrose.........	0.48	1.31	1.02	0.45	0.59	0.21	0.27
Total sugars.....	2.10	3.00	2.19	1.85	1.48	0.79	0.99
Starch and dextrin	2.43	3.23	3.27	0.99	2.03	0.85	0.26
Total carbohydrates........	4.53	6.23	5.46	2.84	3.51	1.64	1.25

TABLE IV

NITROGENOUS FRACTIONS OF WHOLE STEMS EXPRESSED AS PERCENTAGE OF DRY MATTER

	PERCENTAGE						
	APRIL 26	MAY 5			MAY 5		
	INITIAL PLANTS	MINUS-N			PLUS-NO_3		
		55° F.	70° F.	95° F.	55° F.	70° F.	95° F.
Total nitrate-free N........	0.670	0.490	0.655	0.770	0.806	1.211	0.808
Protein N.......	0.453	0.266	0.405	0.394	0.494	0.393	0.377
Nitrate-free soluble N.........	0.217	0.224	0.250	0.376	0.312	0.818	0.431
Basic N.........	0.077	0.060	0.083	0.041	0.079	0.268	0.050
Amino N........	0.150	0.110	0.169	0.225	0.127	0.475	0.136
Amide N........	0.007	0.020	0.014	0.033	0.084	0.053	0.181
Other N........	−0.017	0.034	−0.016	0.077	0.022	0.022	0.064
Nitrate N.......	None	None	None	None	0.464	0.519	0.622
Total N.........	0.670	0.490	0.655	0.770	1.270	1.730	1.430

TABLE V

NITROGENOUS FRACTIONS OF WHOLE STEMS EXPRESSED AS PERCENTAGE OF GREEN MATTER

	PERCENTAGE						
	APRIL 26	MAY 5			MAY 5		
	INITIAL PLANTS	MINUS-N			PLUS-NO_3		
		55° F.	70° F.	95° F.	55° F.	70° F.	95° F.
Total nitrate-free N........	0.093	0.072	0.089	0.097	0.098	0.104	0.081
Protein N.......	0.063	0.039	0.055	0.050	0.060	0.034	0.038
Nitrate-free soluble N.........	0.030	0.033	0.034	0.047	0.038	0.070	0.043
Basic N.........	0.011	0.009	0.011	0.005	0.010	0.023	0.005
Amino N........	0.021	0.016	0.023	0.028	0.015	0.041	0.014
Amide N........	0.001	0.003	0.002	0.004	0.010	0.004	0.018
Other N........	−0.003	0.005	−0.002	0.010	0.003	0.002	0.006
Nitrate N.......	None	None	None	None	0.057	0.045	0.062
Total N.........	0.093	0.072	0.089	0.097	0.155	0.149	0.143

Responses of plants at 55° F.

1. Plants deficient in nitrates.—During the ten-day period at 55° F. the plants with continuous minus-N nutrient treatment did not noticeably increase in volume (fig. 1) but definitely increased in concentration of carbohydrates (tables II, III). There being no external nitrogen supply, there necessarily occurred a decrease in percentage of nitrogen, especially as expressed on a dry weight basis (table IV). Microscopic examination (9) confirmed these results, as shown by the fact that starch was present even in the extreme tip of the stem.

TABLE VI

CHLOROPHYLL IN LEAF BLADES OF TOMATO

	April 26	May 5			May 5		
	Initial plants	Minus-Nitrogen			Plus-Nitrate		
		55° F.	70° F.	95° F.	55° F.	70° F.	95° F.
As percentage of green matter...	0.048	0.033	0.054	0.048	0.039	0.099	0.078
Relative values*.	48	33	55	48	39	100	79

* Calculated using the plus-nitrate 70° F. plants, on May 5, as 100.

During the low temperature treatment the leaves became lighter yellow, and there was loss of chlorophyll (table VI) and apparently destruction of chloroplasts (9). The stems and veins, however, exhibited a very heavy purplish red anthocyanin pigmentation.

2. Plants which received the complete nutrient solution.—Nitrate absorption was apparently instantaneous (9), and in about five hours nitrates were present in all parts of the plant. Translocation seemed therefore to be a little slower than at 70° F. The 55° plants both with and without nitrates gradually decreased in capacity for nitrate reduction (9), although, as may be seen from a comparison of figures 1 and 2, addition of nitrates materially increased the leaf area and increased slightly the height of the plants. Growth with nitrates at 55°, however, was extremely slow. At 70° there was a noticeable response after 36 hours, but only after four or five days at 55° F.

The increase in leaf area of the plus-NO_3, 55° plants was not accompanied by an increase in green color. On the contrary there was loss in concentration of chlorophyll (table VI) and disorganization of chloroplasts, although small and poorly developed chloroplasts appeared in the small apical leaves. These plants, like the minus-N treated individuals, had stiff woody stems, were yellow and heavily tinged with purple.

Associated with limited growth and nitrate assimilation there was, after ten days of 55° treatment, a much lower concentration of carbohydrates than in the minus-N plants at the same temperature (tables II, III).

Organic nitrogen was also higher, especially on a dry weight basis (table IV), and with increase in volume (fig. 2) there must have been increase in total organic nitrogen or there would otherwise have been a loss in percentage as expressed on a green weight basis. That this did not occur is indicated in table V and by the fact that on a green weight basis total organic nitrogen in the roots, stems, petioles, and blades was respectively 0.132, 0.098, 0.097, and 0.281 per cent at the time of final harvest on May 5, whereas corresponding figures for the *initial* plants on April 26 were 0.126, 0.093, 0.058, and 0.266.

RESPONSES OF PLANTS AT 95° F.

1. PLANTS DEFICIENT IN NITRATES.—In less than eight hours after the plants had been subjected to a temperature of 95° F., the petioles, except those of the two lower leaves, became nearly vertical in position and formed an acute angle with the stem axis. This is clearly shown in figure 1. (At 55° and 70° the petioles were more nearly at right angles to the stem and almost horizontal in position.)

During the ten-day period at 95° there was a marked decrease in carbohydrates (tables II, III). In fact, microscopic examination (9), only 36 hours after shifting to the 95° chamber, showed that the amount of starch had materially decreased in the upper 4 cm. of stem tissue. Accompanying the decrease in carbohydrates there was a marked increase in concentration of total and soluble organic nitrogen, as expressed on a dry weight basis (table IV).

The 95° plants, even with no external nitrogen supply, increased noticeably in volume (fig. 1). On a green weight basis, therefore,

there was no increase in concentration of nitrogen (table V). It is significant, however, that without added nitrogen, and associated with increase in volume of the plant, there occurred a distinct decrease in protein and corresponding increase in soluble organic nitrogen (tables IV, V), especially in *other*, amino, and amide nitrogen.

After three or four days at 95°, accompanying the proteolytic response already recorded, the plants turned somewhat darker green. This was soon followed by injury to the chloroplasts (9), however, and when harvested for analysis on May 5, almost the entire plant was yellowish, practically free of anthocyanin pigments, and as low in chlorophyll as the initial plants (table VI).

2. PLANTS WHICH RECEIVED THE COMPLETE NUTRIENT SOLUTION. —It is apparent from figure 2 that these plants responded similarly to the minus-N treated plants at the same temperature with respect to the acute angle arrangement of the petiole and stem. This response became apparent in both groups of plants at about the same time. Further discussion of this point will be found in a paper by ECKERSON (9).

Application of nitrates was followed immediately by their absorption, and nitrate was found in all parts of the plant in a little less than four hours (9) after application. Nitrate reduction (9) also was extremely rapid during the first few hours at 95° F. After that period reducase activity apparently gradually dropped, until on May 5 (after ten days) there was practically no reducase (9) in either plus-NO_3 or minus-N treated plants. This response was clearly reflected externally. In less than 24 hours after nitrate application, the foliage was distinctly darker green than that of any other groups of plants in these experiments; and for three or four days there was a continued increase in stem height and leaf area that far exceeded in rate the response of the plus-NO_3 plants at 70° F. This rapid growth of the plants in the 95° chamber was correlated with decrease in carbohydrate content, especially in the upper half of the plants, and with increase in amino acids and asparagine (9).

After four or five days at 95°, however, the growth rate became relatively slow. As may be seen from tables II and III, there was an extreme decline in carbohydrates. These data, however, are for whole stems. The upper halves of the plants (9) were practically

devoid of starch on May 5. Also on that date a few of the plants (which were not employed for analysis) were dead at the stem tips. The upper leaves, which were very dark green during the first few days, were also rapidly turning yellow. At the time of final harvest the chlorophyll content of aliquots of blades was lower than that found in the plus-NO_3 group at 70° (table VI).

At the time of analysis on May 5, the stems of the plus-NO_3, 95° plants were surprisingly low in organic nitrogen (tables IV, V). The petioles, however, contained on a green weight basis about four times the percentage of organic nitrogen found on April 26 in the same part of the initial plants, and the blades had about doubled in concentration of nitrate-free nitrogen during that period.

Following the ten-day experimental period and the final harvest for analysis, a few plants were kept in the chamber at 95° F. All those receiving complete nutrient died within two or three days. This was in striking contrast to the minus-N treated plants, which showed no indication of death of stem tips nor of other parts during the same period.

During the ten-day period in the temperature chambers the external appearance of the root systems of the respective series was much the same, and the rate of growth corresponded closely to that of the tops. Toward the end of the period of treatment at 95°, however, there was definite injury to the protoplasm of the fine roots (9); but no injury was apparent externally and the roots did not die until several days following the termination of these experiments.

Discussion

Significance of results

The plants of these experiments were subjected to practically constant temperature conditions, and humidity was maintained at approximately 85 per cent in each chamber.[2] Sunlight and carbon dioxide supply were variable but at any given time were essentially the same for all the temperature treatments. The responses of the plants at 55°, 70°, and 95° F. are therefore strictly comparable.

[2] Although the relative humidity was maintained at 85 per cent, the vapor pressure deficit of course varied at each of the three temperatures. Effects of relative humidity and of vapor pressure deficit will be discussed in a paper soon to appear.

Under the same conditions of nutrient treatment, differences observed in growth and metabolism were brought about solely as the result of differences in temperature, although obviously the response of the plants was influenced by all factors of their environment. It should be emphasized, however, that the respective temperatures imposed were continuous night and day. During the night, plants are commonly subjected to temperatures ranging lower than during the day. No attempt was made to duplicate such conditions.

Before discussing these results it would seem pertinent to recall certain relationships between temperature, carbon dioxide, and light. At the highest temperature employed (95° F.), the comparatively low light intensity of the glass inclosed chambers was undoubtedly a limiting factor in carbon dioxide assimilation (14, 32). Yet if more light had been supplied in an attempt to obtain maximal assimilation at 95°, it would also have been necessary to increase materially the concentration of atmospheric carbon dioxide (14, 32). Even if this had been accomplished, however, the factor of increased "inactivation" (14, 32) of the plant at higher temperatures would still have been impossible of elimination, as at 95° there was (table VI) definite injury to protoplasm and especially to chloroplasts (9).

Carbohydrates

The process of protein synthesis from nitrates necessarily involves utilization of carbohydrates resulting in a decrease of those in storage, unless the amount is supplied by new synthesis. But if tomato plants already containing protein reserves are grown with no external nitrogen supply, carbohydrates in the plant will increase, provided the rate of carbon dioxide assimilation exceeds that of respiration, or will decrease if respiration predominates over photosynthesis.

At 55° with minus-N treatment there was a definite increase in carbohydrates in the stems (tables II, III) over that found in the initial plants. A corresponding increase occurred in all other parts of the plant (9), showing beyond question that carbohydrates were synthesized at 55° in greater quantity than they were respired. This occurred even though there was some loss in chlorophyll (table VI) and injury to chloroplasts (9). Likewise at 70° with no external nitrogen supply there was a gain in concentration of carbohydrates

in all parts of the plant but not so great as at 55° F. The somewhat lower carbohydrate content of the series at 70° is not surprising. It has already been pointed out that light and carbon dioxide were limiting factors to maximal assimilation at 70°, whereas respiration was undoubtedly accelerated by the higher temperature without serious limitation by any factor (14, 32). At 95° with minus-N treatment, it is clearly apparent (tables II, III) that there was a much greater loss of carbohydrates through respiration than was compensated for by photosynthesis.

No work seems to have been done by others on effects of temperature on the carbohydrate content of plants grown with no external nitrogen supply. The results of these experiments show beyond question, however, that the lower temperature of 55° resulted in carbohydrate accumulation in the tomato plant; whereas at 95° there was a decrease in carbohydrates. It is emphasized also that these effects of temperature were not the result of prolonged treatments. The total duration of the entire temperature treatment was only ten days in each case, and noticeable changes in carbohydrate content (9) occurred within a few hours after shifting the plants to the respective temperatures.

The proportion of carbohydrate fractions as determined (tables II, III) varied somewhat, yet the results (15, 20) seem to indicate as usual that in the tomato, starch is an important storage form, and that it accumulated with increase in other carbohydrates. That starch is not stored in all species of plants will be pointed out elsewhere.

Reutilization of protein

The plants grown with no external nitrogen supply furnished information on carbohydrate metabolism that could not have been obtained had they been utilizing carbohydrates in the synthesis of proteins from nitrate. These plants are also of additional interpretative value in that any effects of temperature on nitrogen metabolism are limited strictly to effects on organic nitrogen already present in the plant. The situation is not complicated by nitrate assimilation and the consequent presence of newly synthesized nitrogenous compounds.

Figure 1 shows that the plants at 95° with no added nitrogen in the nutrient solution increased materially in volume as compared with the initial plants and with the plants at 55° F. They likewise turned darker green, although later, with injury to chloroplasts (9), and became yellowish. Associated with this growth there must obviously have been reutilization of organic nitrogen. Tables IV and V show this to be the case, since there was a definite increase in the simpler, more mobile forms of organic nitrogen as amino, amide, and *other* nitrogen, and a corresponding decrease in proteins and basic nitrogen, the latter fraction consisting partly of polypeptides.

The record of this response is not new (18, 20, 21), nor is it in any sense peculiarly related to high temperature alone. A nitrogen deficient tomato plant high in carbohydrate reserves, with yellowish green foliage, may be made to grow and turn dark green if the concentration of its contained carbohydrates is reduced and the percentage of its organic nitrogen thereby relatively increased, by subjecting the plant to shading or to a short photoperiod (18). That the high temperature plants of these experiments did decrease in carbohydrates to a great degree has already been indicated (tables II, III).

With the decreased light intensity of the temperature chambers, there was in the plants lacking nitrate at 70° some evidence of growth in the stem tips and terminal leaves. In these organs, however, carbohydrates were much lower (9) than is indicated by the average carbohydrate content of whole stems of the same plants, as determined macrochemically (tables II, III). At 55° the plants lacking nitrates made no noticeable growth as compared with those of the initial series, even though there was a considerable increase in concentration of amide and *other* nitrogen. This increase, however, may have been one of the results accompanying injury of the protoplasm and of the chloroplasts (9) at 55° (table VI), rather than proteolysis similar to that occurring at 70° and 95° F. Increase in amide and other nitrogen seems often to occur under somewhat comparable conditions (3, 4, 18, 20).

Absorption and translocation of nitrate

Absorption of nitrate was not a limiting factor at any of the temperatures employed (9). As nearly as could be determined, there

appeared to be immediate absorption of nitrate by the root hairs. On the basis of nitrate appearance within the roots, no difference in permeability could be detected at temperatures of 55°, 70°, and 95° F. Different results might have been obtained with more extreme temperatures (7, 26, 36). The rate of translocation through the root system and top was a little slower at 55° than at 70°, however, and in turn nitrates were found in all parts of the plants at 95° a little sooner than at 70° (9). Yet five hours after nitrates were added to the sand in which the plants were growing, there was an abundance of nitrate in all parts of the plants, even at 55°; and when harvested for analysis on May 5, the plants which were subjected to the lowest temperature had on a green weight basis a higher concentration of nitrate than the plants at 70° (tables IV, V). In the case of asparagus (19), it has been pointed out that at a temperature of 45° F. the absorption of nitrate was not limited but the same temperature inhibited its assimilation.

The plants at 55° made little growth (fig. 2), and as will be shown presently, used or assimilated little nitrate. In about five hours, therefore, they became filled with nitrate to maximum capacity and removed very little more from the culture medium, as was evidenced by no tendency toward pH change (12, 13) of the residual nutrient solution. On the basis of nitrate removed from the culture solution, it might be said that at 55° temperature limited absorption; yet obviously such an interpretation is inaccurate, especially if it implies that the root system is impermeable to nitrate.

In connection with absorption of nitrate, plants of the various temperature and nutrient treatments were analyzed for calcium, magnesium, potassium, phosphorus, and sulphur. The plants without nitrate had almost exactly the same concentration of each element at 55°, 70°, and 95° F. Likewise the nitrate supplied plants at 55°, which were assimilating little nitrate, were only slightly higher. All of these elements (8, 16, 21, 23), however, including sulphur in certain forms (22), are known to be required in abundance in rapidly growing tissues. It seems logical, therefore, that there was found in the vigorously growing plants with complete nutrient at 70° a considerably higher percentage of each of these elements than was present in comparatively inactive plants. The plants which for a time

were making vigorous growth at 95° with nitrate, were at the end of ten days only a little lower in these elements than the nitrate supplied plants at 70° F.

Nitrate assimilation

It has been indicated that absorption and translocation of nitrate were not limiting factors at any of the temperatures employed in these experiments. Nitrates were present in high concentration in all parts of the plants (9) at each temperature. The high percentage of nitrate nitrogen in the stems is reported in tables III and IV. Nitrates in themselves, however, will not produce growth (18, 20). They are not an essential part of the living protoplasm of plants, but rather represent an excess of nutrient material not yet metabolized.

On the basis of growth response alone, it is clearly apparent from figure 2 that there was, at the respective temperatures employed, a marked difference in ability to utilize nitrate. The greatest growth was made at 70°, and in these plants Eckerson (9) found a consistently high concentration of nitrate reducing material. Likewise in this group of plants, accompanying rapid synthesis of amino acids (9), there necessarily occurred oxidation or decrease in carbohydrates (tables II, III). Associated with these changes there was, in the first 48 hours after the initial addition of nitrate to the nutrient medium, noticeable growth of the plant as a whole and change in color of foliage from yellowish to darker green, until finally on May 5, ten days later, the plants had approximately doubled the chlorophyll content of the blades (table VI) and had become materially lower in percentage of carbohydrates but comparatively high in content of all determined forms of organic nitrogen. Correlated with these internal changes the plants grew vigorously as usual (15, 20, 21), and the new growth was comparatively soft and succulent.

The plants at 55° decreased rapidly in content of nitrate reducing material, until finally at the end of ten days reducase was practically absent (9). It is apparent (fig. 2), however, that added nitrate at 55° resulted in increased growth, although growth and assimilation of nitrate were extremely slow as compared with that at 70° F. At the lower temperature there was scarcely any perceptible growth during the first five days, and after this period the rate of growth

was even slower. Associated with this great retardation in growth there was little, if any, nitrate assimilation. Such limited assimilation of nitrate at 55° is further indicated by the somewhat higher concentration of organic nitrogen in the stems (tables IV, V) and other plant parts of those supplied with nitrate as compared with the initial plants and comparable plants lacking nitrate. The notably high concentration of amide, however, may not have been entirely a synthetic product (3, 4, 20).

Accompanying limited assimilation of nitrate at 55° there necessarily occurred some decrease in reserve carbohydrate content, and the nitrate supplied plants at this temperature, although remaining extremely high in carbohydrates, showed a definitely lower concentration of sugars and starch (tables II, III) than the initial plants or the series without nitrate at the same temperature.

There was also exhibited externally the usual symptoms (15, 20) characteristic of plants high in carbohydrates but deficient in proteins. Growth was slow and stems were hard, woody, and heavily tinged with the bluish purple of anthocyanins; the foliage was conspicuously yellow, probably partly because of protein deficiency but also because of gradual (9) decrease in chlorophyll (table VI) under the low temperature treatment.

In connection with these responses, the work of ROSA (29) is of particular interest. He grew tomato plants in soil in a "warm greenhouse" and others in a cold-frame where they were "exposed to temperatures near freezing during the night and to full sunlight during the day." The plants in the cold-frame apparently responded much as did those just described. He made no nitrogen determinations, but found that plants in the cold-frame were more than three times as high in starch as those in the warm greenhouse. In cabbage, ROSA found that similar treatment resulted in accumulation of carbohydrates, these consisting of sugars and pentosans rather than of starch.

There seem to be few references which deal directly with the effects of temperature on nitrate assimilation, although WALSTER (34) found that carbohydrates accumulated in much higher concentrations in barley plants that were grown in a greenhouse at approximately 15° C. than was the case in plants subjected to a greenhouse

temperature of about 20° C. He ran nitrogen analyses, but only of the tops, which leaves doubt as to the interpretation of his results, since in the Gramineae it has been reported (30) that nitrate reduction occurs mainly in the roots. In the asparagus plant also nitrate assimilation is limited almost exclusively to the fine roots, and assimilation of nitrate in these organs (19) apparently does not occur at temperatures below 10° C.

Effects of temperature will, of course, vary with the species and variety of plants employed. Yet experiments are consistent in showing that moderately low temperature results in such typical responses as hastened or accentuated tuberization in potato (2); premature seeding in plants such as beet (5), cabbage (29), cauliflower (28), and celery (27); and earlier grain development of barley (34). Such responses seem typically[3] to occur (15, 20) when plants are accumulating carbohydrates, not when there is rapid assimilation of nitrate and consequent decrease in carbohydrates. Decreased nitrate elaboration, however, is only one reason for increase in concentration of carbohydrates. It will be recalled that tomato plants at 55° with no nitrate supply gained materially in percentage and amount of sugars and starch, apparently because photosynthetic activity at that temperature predominated over respiration.

There is little pertinent information available on effects of higher temperatures on the growth and composition of plants. In connection with the responses of tomato at 95°, it may again be pointed out that under the conditions of these experiments the rate of respiration exceeded carbohydrate synthesis. During the early stages of growth

[3] PLATENIUS (27) demonstrated conclusively that low temperature hastened seeding in celery, but he did not find consistent differences in composition of seeders and non-seeders. He analyzed whole plants on the assumption that "all parts undergo similar changes in composition under each treatment." Such an assumption is obviously not tenable, and too, vegetative and reproductive plants have very different proportions of blade, petiole, and storage root tissue; and in a given plant each of these parts is extremely different in composition. Kind or quality of growth seems (15, 20) to be intimately associated with quality of or percentage composition. The percentage composition of a heterogeneous whole-plant aliquot may be of doubtful significance, and absolute amounts per whole plant are correlated with plant size, not quality.

Starch is only one (6) of the many non-nitrogenous storage reserves in plants; and although PLATENIUS analyzed for sugars as well as for starch, he made no determinations of mannite, which is notably high in celery (24, 25, 35).

at 95°, when the reserve carbohydrate content of the plants was still high (tables II, III), nitrate reduction (9) and growth were more vigorous and rapid than at 70°; but after the higher temperature had been imposed upon the plants for ten days, their reducase content had dropped to practically nothing (9). As they synthesized organic nitrogen, the nitrate supplied plants of course lost carbohydrates (tables II, III) more rapidly than those with no nitrate.

At the end of the ten-day period occasional plants of the complete nutrient series at 95° were dead at the tips (and were discarded). Examination of other plants from this series showed that in the upper halves of the tops carbohydrates were extremely low (9), and had decreased very greatly throughout the stems as a whole (tables II, III). The plants obviously lost chlorophyll, as analyses ultimately indicated (table VI). In the latest stages of growth the plants were weakly vegetative, and very soft and succulent, a condition that frequently (15, 18, 20, 21) accompanies extreme deficiency in utilizable carbohydrates.

At the time of final harvest for analysis, the stems (tables IV, V) were not high in organic nitrogen; but, as already mentioned, apparently there was translocation of organic nitrogen to other organs of the plant, principally to the petioles. In both stems and petioles there was associated with carbohydrate deficiency the usual (3, 4, 18, 20) marked decrease in percentage of protein and increase in the less complex fractions, especially amide and *other* nitrogen (tables IV, V).

Summary

Tomato plants were grown in sand culture in glass inclosed chambers and humidity was maintained at 85 per cent in all cases. Sunlight and carbon dioxide supply varied but at any given time were the same for each temperature treatment. The following relative *differences* which occurred in growth and metabolism at 55°, 70°, and 95° F. were brought about as the result of differences in temperature.

1. TEMPERATURE 55° F. (13° C.).—At this temperature carbohydrates, especially starch, accumulated in large quantities, indicating beyond question that assimilation of carbon dioxide exceeded respiration.

Nitrate was absorbed instantaneously, and in about five hours

was present in high concentration throughout the plant and remained high.

Nitrate was assimilated (synthesized to organic nitrogen) very slowly and the plants were very low in nitrate reducing material (9). Accordingly, carbohydrates were high even in nitrate supplied plants, as there was little utilization of carbohydrates in protein synthesis.

Externally the plants were yellowish green, owing to a low chlorophyll content and comparatively few and poorly developed chloroplasts. The veins and stems were purplish, from the presence of anthocyanins. The stems were hard and woody and the cell walls thick. This was true even of plants supplied with nitrate, although these conditions were accentuated in plants lacking nitrate.

2. TEMPERATURE 70° F. (21° C.).—Plants lacking an external nitrogen supply were not so high in carbohydrates as comparable plants at 55° F., and exhibited symptoms of protein deficiency in less degree.

Nitrate absorption was apparently instantaneous and translocation of nitrate was slightly more rapid than at 55° F.

The plants were high in nitrate reducing material (9) and assimilation of nitrate was rapid, hence there was a comparatively high concentration of carbohydrates in plants lacking nitrate as compared with those receiving nitrate.

Plants supplied with a complete nutrient solution were moderately high in carbohydrates, contained an abundance of elaborated nitrogen, were dark green, rather succulent, and grew vigorously.

3. TEMPERATURE 95° F. (35° C.).—Plants with no external nitrogen supply as well as those with complete nutrient rapidly decreased in carbohydrates, indicating that respiration exceeded carbon dioxide assimilation at this temperature.[4]

Accompanying decrease in concentrations of carbohydrates, there was a breaking down of complex proteins to simpler forms of organic nitrogen, accelerated growth for a few days, and death of the plants.

Nitrate absorption was apparently instantaneous and translocation of nitrate was a little more rapid than at 55° or 70° F.

[4] With increase in temperature, limiting effects of carbon dioxide supply and light intensity are accentuated, page 47.

At first nitrate assimilation and reducase activity (9) were extremely high and the external response occurred much sooner than at 70°, but after four or five days nitrate assimilation practically ceased and the nitrate supplied plants grew much more slowly than at 70° F.

Because of carbohydrate utilization in protein synthesis, as well as in respiration, the plants which received nitrate lost carbohydrates more rapidly and died sooner than those with no external nitrogen supply.

During the early stages of 95° treatment, the plants receiving a complete nutrient solution grew rapidly and were dark green, although spindling. After a few days there was disintegration of chloroplasts, the newly developed leaves contained few and small chloroplasts (9), and the plants as a whole were soft, yellowish green, mottled, and practically free of anthocyanin pigmentation.

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A CLASSIFICATION OF THE ALGAE BASED ON EVOLUTIONARY DEVELOPMENT, WITH SPECIAL REFERENCE TO PIGMENTATION

JOSEPHINE E. TILDEN

(WITH PLATE I)

Introduction

In early treatises on algae these plants were grouped according to color; later this was considered to be an artificial method. It now seems, however, that the pigments characteristic of the various types must have been of the most vital importance in the development and advancement of these organisms; therefore a classification with pigmentation as a basis is entirely logical. Accordingly such names for the classes of algae as have reference to color should be retained and a new name adopted to include the several types of algae in which the chromatophore has a golden yellow, greenish yellow, or yellowish brown hue. PASCHER'S (35) term "Chrysophyceae" seems very appropriate, unless it should be proved in the future that the yellowish color is due in all cases to an excess of xanthophyll, when it would seem better to substitute the name "Xanthophyceae," recently suggested by ALLORGE (1) to take the place of "Heterocontae."

The special pigmentation character belonging to each group of algae must be a heritable character of high permanence; evidently it was definitely established in the organism during its early history, for certain types of the five races of algae are today found growing side by side under identical environments.

The writer (TILDEN 41, 42) believes that the primary factor in the differentiation of these groups was a gradual increase in the quantity, or change in the quality, through incalculable periods of time, of solar energy, which reached the organism after its passage through the two overlying media, air and water. The individual pigments and groups of pigments present in the cells of living algae may be compared to index fossils in beds of sediment. They were succes-

sively developed in response to an environment of gradually changing radiant energy, and they furnish in themselves infallible evidence of sequence in time.

It is the purpose of this paper to call attention to the fact that, whatever may be our conception of the prototype of algal organisms, the working basis for new investigation in this important group of plants lies in the data we have at hand concerning the plants which are in existence today. From these facts alone we must make our interpretation of conditions in the past and develop a plan for attacking the problems of the future. Toward this end, a new method of arranging and classifying the mass of details with which all students of algae are familiar is herewith submitted, in the hope that it may be of value in indicating new lines of research.

The table presented in plate I embodies the views of the writer with regard to the evolution of the algae. Algal "periods" have been inserted in the geological time scale, and corresponding to these periods is shown the group of pigments characteristic of each class of algae. It would seem safe to place the beginning of the algae in general in the Archaeozoic. The position of each class indicates its possible or probable point of origin. It must be understood that types of living algae only are included.

Phylogenetic relationships as indicated by cytological evidences, pigments, and food reserves

In building up a classification of the algae, it seems logical to begin with the group of living forms presenting the simplest type of cell structure.

CYANOPHYCEAE

The work of PRAT (38), MOCKRIDGE (30), and POLJANSKY and PETRUSCHEWSKY (37) has made it clear that the Cyanophycean cell possesses no organized nucleus. POLJANSKY and PETRUSCHEWSKY emphasize the following points in connection with their investigations on species of *Spirulina*, *Oscillatoria*, *Tolypothrix*, and *Gloeotrichia*. The cell is made up of (1) a cellular membrane; (2) a peripheral plasma consisting of protoplasm diffusely saturated with pigments; and (3) a central body (the central colorless portion of the cell) which contains metachromatic granules, stored assimilation products such as glycogen, and a "chromatic substance."

POLJANSKY and PETRUSCHEWSKY do not hold the view that the peripheral plasma should be considered as a limited chromatophore, since the central body is not sharply outlined but seems to change gradually into the peripheral plasma. Nor, in their opinion, is it correct to conclude that the central colorless portion of the cell is a nucleus without a membrane. It is more nearly correct to compare the basal substance of the central body with the cytoplasm of the ordinary cell. On the other hand, there is a substance in the central mass which definitely gives the nuclear reaction and stains with basic dye. The form in which this substance occurs varies from one species to another, appearing as a network, a spongy mass, or as strands stretched out in a longitudinal direction. To this chromatin-like material they have applied the name "chromatic substance." There is no reason to assume that the definite parallel arrangement of the chromatic material, observed during division in some species, is a primitive mitosis. The division of a cell of *Spirulina*, for example, may be characterized as a single constriction of the contents of the cell into two parts, during which process there is developed a new cellular membrane. The chromatic substance forms no chromosomes during its division, but divides by simple fission. The chromatic substance, however, is chemically either identical with, or closely related to, the chromatin of a true nucleus. Supposedly these structures are also phylogenetically homologous.

Cyanophycin granules are particularly abundant in hypnogonidia. At least in the case of *Gloeotrichia*, they are to be considered as inclusion bodies of the peripheral plasma.

Thus a definite picture of the form and structure of a simple cell, or unicellular plant, is gained from the conclusions of POLJANSKY and PETRUSCHEWSKY. The same picture may represent an ideal ancestral organism, for the simplest living algal plants of today (that is, unless reduced or retrograde) probably resemble the ancestral form far more closely than do any of the higher algae.

PIGMENTATION.—It is assumed that the progenitors of the blue-green algae came into being during the period of weakest illumination, the "Cyanophycean period."

Most of the species belonging to the Cyanophyceae are pale, dull, or vivid bluish green in color; a few are rose-violet or brown. These

various tints are due to the presence of certain pigments in the protoplast, of which *phycocyanin*, a blue pigment, is the dominant one. *Phycoerythrin*, a rose-red or violet-red pigment, is more or less abundant in certain species, but it is believed to be entirely lacking in others. Chlorophyll and carotinoid pigments are present, but practically nothing is known of the "chlorophyll-carotinoid" complex of the blue-green algae. No one has been able to establish the chemical formula for any one of their pigments.

Phycocyanin alone, or in association with phycoerythrin, may have as its function the absorbing of the penetrating rays of light and the transferring of them to the chlorophyll and carotinoid, as the centers of the process of photosynthesis. DANILOV (10, 11) investigated the water-soluble pigments of blue-green algae grown in pure culture, and also of red algae collected in the Arctic Ocean. He doubts the protein nature of the pigments and thinks it more probable that they are dissolved in the proteins. KLUGH (20) suggests that the pigment known as phycoerythrin is an optical sensitizer which enables the chlorophyll of the algae possessing it to make use of the radiations which would, without its presence, be extremely ineffective. On the other hand, phycocyanin and phycoerythrin themselves may be as much concerned in photosynthesis as is the chlorophyll-carotinoid combination.

With reference to the presence or absence of the pigments phycocyanin and phycoerythrin, the Cyanophyceae fall into three divisions according to GAIDUKOV (14), BOCAT (3), KYLIN (24, 26), BORESCH (5, 6), ISHIKAWA (18), WILLE (45), and NAKANO and HIGASHI (31). They are as follows:

1. *Blue-green algae containing phycocyanin alone.*—The majority of the blue-green algae contain phycocyanin only. Ordinarily they exhibit a blue-green or Spanish green color:

Oscillatoria tenuis, O. formosa
Phormidium corium, Ph. laminosum var. aeruginea,
Ph. autumnale var. aeruginea
Anabaena sp.

2. *Blue-green algae containing both phycocyanin and phycoerythrin.*—These forms are blue-green or dark olive-green in color:

Oscillatoria sancta, O. okeni, O. limosa, O. amoena, O. caldariorum
Phormidium favosum, Ph. persicinum, Ph. subfuscum, Ph. laminosum var. olivacea-fusca, Ph. autumnale var. olivacea, Ph. retzii var. nigro-violacea
Lyngbya aerugineo-caerulea
Microchaete calotrichoides
Phylloderma sacrum

WILLE (45) made some interesting observations on *Phormidium persicinum*, which he found on the south coast of Norway. His notes, written in 1889, bear out recent findings with respect to these pigments. At a depth of 18 feet an abundance of *Laminaria saccharina* occurred on which were growing some Bryozoan colonies. The empty shells of the Bryozoa contained large numbers of *Ph. persicinum* of a Floridean (Rhodophycean) red color. With the help of the Zeiss absorption spectroscope, he compared the blue-green alga with a red one, *Rhodymenia palmata*, and states that the absorption spectra of *Ph. persicinum* and *R. palmata* are specific, and therefore that *Ph. persicinum* contains genuine phycoerythrin. WILLE concludes that, together with other conditions, this points toward the phylogenetic relation of the Florideae (Rhodophyceae) to the Cyanophyceae. The phycocyanin-containing Cyanophyceae occur only near the surface, while *Ph. persicinum* grows at depths of 18–24 feet or more, where usually only red algae thrive. The substitution of phycoerythrin for phycocyanin at greater depths is apparently connected with the difference in their property of absorbing light.

3. *Blue-green algae containing phycoerythrin alone (or with very little phycocyanin).*—These are the dark olive-brown, light sepia-brown, brownish red, and brownish violet varieties:

Oscillatoria cortiana
Phormidium luridum and its two varieties, fusca and violacea
Microchaete tenera

FOOD RESERVES IN CYANOPHYCEAE.—Glycogen in the blue-green algae appears to function much as starch does in the higher green

plants. The studies of POLJANSKY and PETRUSCHEWSKY reveal several interesting facts concerning this substance. Apparently the prèsence of glycogen is connected with the physiological condition of the alga. It occurs usually in the peripheral plasma. Only a small amount was determined in species of *Gloeotrichia* and *Tolypothrix*, while *Oscillatoria* and *Spirulina* contained a much greater quantity. In *Oscillatoria princeps* it was found to be present in both the central and the peripheral portions of the protoplast.

RHODOPHYCEAE

A long period of time must have elapsed between the Cyanophycean and Rhodophycean periods, in which in turn the blue-green and the red algae constituted the dominant flora of the sea. All that remains today to indicate the transitional forms are a few species of red algae usually designated as the Protoflorideae. The period of Rhodophyceae must have been characterized by a light intensity somewhat corresponding to that at a depth in sea water which the majority of the red algae seem to prefer.

It seems not unlikely that, in some of the mutants of the ancient blue-green algal cell, the scattered masses of chromatic material might have gained in size; that eventually the separate particles might have taken on a rodlike shape; and that gradually these rod-shaped bits might have been collected into a small portion of the cell within a drop of more concentrated and finely granular protoplasm. Such a structure would approach that of a primitive nucleus.

In the Protoflorideae there is a distinct nucleus. ISHIKAWA'S (18) cytological studies on *Porphyra tenera*, however, convince one that he was working with a simple type of nucleus. He considered that the mode of nuclear division might be taken as a primitive mitosis, midway between amitosis and mitosis. In addition to this nucleus ISHIKAWA distinguished a distinct chromatophore. A peculiar pyrenoid appears in the cell and likewise the stored carbohydrate, known as "Floridean starch."

The fresh-water unicellular form so common in greenhouses, *Porphyridium cruentum*, until recently was included under the Cyanophyceae; but a nucleus being discovered in the cell as well as a star-shaped chromatophore, it was transferred to the Protoflorideae.

In all of the members of the Rhodophyceae other than this small group, the nucleus of the cell appears to be of as high a type as that of other plants and animals.

PIGMENTATION.—As in the supposed development of a nucleus, a similar gathering together of the separate pigment particles into a slightly differentiated mass of protoplasm would constitute a simple chromatophore. It has already been shown that there must have been a gradual transition in the phycocyanin-phycoerythrin pigments of the blue-green algae, since we still have the descendants of those algae containing phycocyanin alone, phycocyanin associated with phycoerythrin, and phycoerythrin alone.

Whether the chlorophyll of the red algae is in the form of *a* and *b* has not yet been ascertained, but both carotin and xanthophyll are reported by HANSEN (17), KYLIN (24, 25, 26), PALMER (34), and others. Phycoerythrin, the red pigment, is so abundant that as a general rule it entirely masks the other pigments. Phycocyanin is found to be present in some cases and absent in others, according to KYLIN (24, 26), ISHIKAWA (18), and WILLE (45).

1. *Red algae containing phycocyanin alone.*—In a very small number of species phycocyanin is present and phycoerythrin absent:

Asterocytis ramosa
Batrachospermum vagum, B. testale, B. virgatum

2. *Red algae containing both phycoerythrin and phycocyanin.*—Both pigments are found in numerous species of red algae (KYLIN):

Protoflorideae:
- Bangia fusco-purpurea
- Porphyra tenera, P. hiemalis, P. umbilicalis

Euflorideae:
- Freshwater forms:
  - Batrachospermum dillenii, B. gallaei, B. moniliforme, B. helminthosum
  - Lemanea fluviatilis
- Marine forms:
  - Nemalion multifidum
  - Dumontia filiformis
  - Furcellaria fastigiata
  - Ahnfeltia plicata

Phyllophora membranacea
Chondrus crispus
Ceramium rubrum
Laurencia pinnatifida

3. *Red algae containing phycoerythrin alone.*—In the majority of red algae phycoerythrin is not accompanied by phycocyanin:

Protoflorideae:
Porphyridium cruentum
Euflorideae

ISHIKAWA (18), working on the cytology of *Porphyra tenera*, did not hesitate to state that in his opinion the coexistence of the two sorts of coloring matter proves an intimate relation existing between *Porphyra* and the blue-green algae. He agrees with OKAMURA in believing that *Porphyra* has a close affinity with the Cyanophyceae, having a connecting link in *Porphyridium cruentum.* In general he feels that the evidence strongly favors the view that the Rhodophyceae sprang from a Cyanophycean stock, and that the higher Rhodophyceae (Euflorideae) descended through the Bangiales (Protoflorideae).

FOOD RESERVES IN RHODOPHYCEAE.—KOLKWITZ (21) proved that "Floridean starch," a substance in the form of granules found in the cells of the Rhodophyceae, is mainly an assimilating product, or a reserve material. The granules stain brownish with iodine, but if in any way they are made to swell and are then treated with iodine, they show different colorations. KOLKWITZ found Floridean starch in all the red algae of the North Sea which he examined. He differentiates two types of starch: a *Laurencia* type with light wine-red color, and a *Furcellaria* type of bluish violet tones. In some red algae blue shades appear which are almost identical with the customary starch reaction, but the true shade was never obtained. In future investigations it may be found that Floridean starch consists of different amyloses, or related substances, occurring in various amounts in the different species, thus causing a different coloration with iodine in each case. While it is a fact that small molecules of ordinary starch give variable blue and red tints upon testing, the structure of Floridean starch undoubtedly deviates from that of true starch.

The granules of Floridean starch lie in the cytoplasm, but they begin their formation at the surface of the chromatophore as flat plates which become somewhat conical, the flattened bases remaining in contact with the plastid as long as they continue to grow. Apparently in some cases they are attached to the inner concave surface of the chromatophore. The older granules are free in the protoplasm. OLTMANNS (33) suggests their similarity to pyrenoid-like structures which may be formed without any connection with the chromatophores. CLELAND (8) reports a close association of the pyrenoid of *Nemalion* with the formation of Floridean starch. The findings therefore indicate that granules of some material which contains carbohydrates are present, and that this material constitutes the assimilation product of the Rhodophyceae.

Another substance which occasionally may be observed in the red algae (as in *Laurencia* and *Plocamium*, for example) is oil. No particular study has been made in this field, and therefore no additional facts are at hand.

PHAEOPHYCEAE

It is assumed that new conditions of illumination were ushered in during the Phaeophycean period, and that possibly shallow seas, gradually sloping shores, and numerous tide-pools were in existence. Penetration of the radiance of the sun attained fuller power but by no means equaled that of the present time.

There are no transitional forms to make it possible to understand what happened to cause development of the new type of marine plants, other than the changes in solar energy. The brown seaweeds, as we know them, range from simple filaments to massive treelike structures.

PIGMENTATION.—Neither phycocyanin nor phycoerythrin occurs in the brown algae. The Phaeophycean algae living today have a peculiar olive-brown, olive-green, or brownish green color, and are characterized by a brown pigment which was announced by SORBY (40) in 1873. He proposed for it the name of "fucoxanthin."

WILLSTÄTTER and PAGE (46) completed the proof that chlorophyll is contained in the brown algae. WILLSTÄTTER and STOLL (47) state that the chlorophyll of these plants shows a remarkable deviation from that of land plants as well as from that of the green algae.

It consists almost exclusively of the *a* component, while only traces of chlorophyll *b* are to be observed, at most 5 per cent. They state: "The chlorophyll component *a*, isolated from the Fucoideae by WILLSTÄTTER and PAGE, agrees, in its magnesium and phytol content and in the composition of the nitrogen-containing cleavage products, with the pigment from other plants."

The brown algae contain a much greater amount of yellow pigment than do the green algae and land plants. The chlorophyll is masked by them because the yellow pigments predominate quantitatively. "The molecular ratio of the green to the yellow pigments is here about 1:1 instead of 3 to 5:1 as in many land plants" (47).

TSWETT (43) proved that three carotinoids are present in these seaweeds: carotin, xanthophyll, and fucoxanthin. WILLSTÄTTER and PAGE determined the quantitative distribution of the carotinoids. Their formulas are given on plate I. Of the three nitrogen-free pigments, fucoxanthin is richer in oxygen than the other carotinoids, its formula being $C_{40}H_{54}O_6$.

FOOD RESERVES IN PHAEOPHYCEAE.—KYLIN (27, 28) suggested the name "fucosan" for certain vacuoles or vesicles found in the cells of brown algae, particularly in the assimilation tissue and in the reproductive organs. According to him these vesicles contain a substance related to the tannins.

In all brown algae mannite is present as a reserve material. OLTMANNS (32) remarked: "It is, therefore, possible that the supposed phloroglucin plays only a secondary part; and evidence is increasing that the assimilation products of the Brown Algae are carbohydrates." KYLIN has proved this to be a fact. He has found monosaccharides (dextrose and levulose) in the Laminarieae and the Fucaceae. These sugars, as in the higher plants, are soon transformed into polysaccharides and are demonstrable in small quantities.

In the Laminarieae, "laminarines," a group of polysaccharides, are found stored, but the individual sugars have not been investigated as yet. In *Laminaria saccharina* and *L. digitata*, laminarines form up to 35 per cent of the dried material by weight; *Fucus serratus*

contains 19 per cent; *F. vesiculosus* and *Ascophyllum* only 7 per cent. In the last species the carbohydrates are substituted in considerable quantities by fat. According to MANGENOT (29), oil is formed by phaeoplasts in some of the brown algae.

The metabolism of the assimilation material of the Phaeophyceae, therefore, is completed in very much the same manner as in the green plants. The monosaccharides produced have been proved by KYLIN to form disaccharides, which are changed into polysaccharides. This substance is not starch, however.

CHRYSOPHYCEAE

In the Chrysophycean period there apparently came into existence great numbers of new types, most of them unicellular, motile during at least a part of their life cycle, microscopic in size, and far more simple in structure than any of the forms immediately preceding them. The present day representatives of this group indeed manifest a strong resemblance to the reproductive bodies of the Phaeophycean algae, and, when ciliated (as they are in most of the subclasses) they exhibit the laterally inserted, unequal cilia characterizing that class. They contain a distinct nucleus, one or more brownish or yellowish chromatophores, and usually one to several pulsating vacuoles.

PIGMENTATION.—In the classification introduced in the present paper, a number of different groups (WEST and FRITSCH 44) have been placed as subclasses under the general class name Chrysophyceae. The algae belonging to these several subclasses are in many cases very abundant at the present time, some in salt water, some in fresh water, and many in both habitats. They all agree in having golden brown or yellowish green chromatophores. Apparently the chlorophyll-carotinoid combination remains, fucoxanthin has disappeared, while an excess of xanthophyll gives to the chromatophore the characteristic golden tint. Since most of the forms are unicellular, the study of their pigments has so far been difficult or impossible, but it is probably safe to state that the Chrysophycean forms now living are characterized by a chromatophore possessing an excess of xanthophyll.

PIGMENTATION AND FOOD RESERVES IN THE VARIOUS SUBCLASSES OF THE CHRYSOPHYCEAE.—

TRIBONEMEAE

This group includes filamentous, unicellular, and coenocytic algae. The cells contain one, two, several, or many chromatophores, usually devoid of pyrenoids. The color is a peculiar yellow-green and is due to an excess of xanthophyll. Such chromatophores characterize all the Tribonemeae and are invariably associated with an absence of starch.

These plants, without exception, build through photosynthesis oil or fatlike droplets which are assumed to be formed from carbohydrates.

DIATOMEAE

The diatoms possess various kinds of chromatophores: small and discoid, large and platelike, band-shaped, and lobed. They are usually yellow or golden brown in color, very rarely green. While they contain the usual types of pigments, there is a preponderance of a brown substance which may or may not be xanthophyll (BEIJERINCK 2).

The products of photosynthesis accumulate as drops of fatty oil which are usually conspicuous in the cells under favorable conditions of nutrition. During rapid growth the oil decreases in amount, but increases again as growth becomes less active. The oil is usually observed outside the chromatophores, but it has also been found within them.

PERIDINEAE

Dark yellow or brown chromatophores, usually numerous and discoid, are characteristic of these algae.

Reserve food is stored as oil and in some cases as starch.

CRYPTOMONADINEAE

As a rule there are two large brown parietal chromatophores, mostly apposed to the dorsal and ventral margins of the organism. Rarely the chromatophores are discoid and numerous. Nothing is known about the nature of the pigments, but various shades in addition to brown are seen, as red, olive-green, and blue-green.

After active photosynthesis the products are stored as solid discoid granules which in some species seem to be a form of starch.

Chrysomonadineae

This subclass is characterized by the possession of chromatophores of a golden yellow or brown color, but when the organisms are living in a habitat rich in organic substances, the chromatophores become greenish in tint.

The products of photosynthesis are stored as oil and leucosin. Starch is not known to occur. Leucosin has not been thoroughly investigated, but it is thought to be a carbohydrate. It occurs as colorless, highly refractive, usually rounded lumps (Pascher 36).

Chloromonadineae

The numerous discoid chromatophores exhibit a peculiar bright green tint which is due to the presence of xanthophyll in greater or less amount.

Food reserves are stored as oil.

Euglenineae

The chromatophores exhibit a pure green color. They are discoid, lobed, or band-shaped, and there may be several or many in an organism.

In many species fat globules act as a food reserve. Starch is never demonstrable, but a peculiar substance, named paramylon by Gottlieb (16) because its composition resembled that of starch, is common. From tests it may be concluded that paramylon is a polysaccharide. It occurs in the form of large granules of a characteristic shape.

Chlorophyceae

The green algae are characterized by bright green chromatophores, or "chloroplastids," developed in response to the bright light of the sun which in all probability reached the earth in its full intensity for the first time in the Chlorophycean period.

Pigmentation.—As in the higher plants, the chloroplastids of the green algae contain but the four pigments, chlorophyll *a*, chlorophyll *b*, carotin, and xanthophyll, usually designated the "chlorophyll-carotinoid" combination.

Willstätter and Stoll (47) investigated the green alga, *Ulva lactuca*, for comparison with the brown algae. They found the

chlorophyll relatively rich in the *b* component. They state: "The ratio of the chlorophyll components in this seaweed deviates, though not far from the average value (about 3), towards a smaller value, just as in the case of the Phaeophyceae." Carotin and xanthophyll are present in relatively greater proportions than in the higher plants, although not to the same extent as in the brown algae.

Food reserves in orders of the Chlorophyceae.—Siphonales

This group of marine algae evidently is the most nearly related to the next older one, the Chrysophyceae. In this respect it is interesting to note that, in certain members of this order, the stored reserve material consists of globules of oil, suggesting the various subclasses of the yellow-green algae. *Vaucheria*, for instance, produces large amounts of oil in its coenocytic body as well as in the resting zygote. Some investigators think that the oil drops hang on the outside of the chloroplastid, from which they appear to have been exuded. At any rate the oil is usually observed outside the chromatophore. It was made evident by Borodin (7) that the oil drops arise from assimilation. The oil increases in quantity in the light and decreases in the dark. It is thought that the formation of oil is preceded by a carbohydrate. The same thing may be true for the oil of *Bryopsis* and other members of the Siphonales. While *Vaucheria* produces oil (Borodin 7), in another genus (*Dichotomosiphon*) belonging to the same family the stored reserve material consists of starch.

Protein is undoubtedly stored in amorphous form in many cases in the algae, as in the higher plants. Commonly it appears in crystalloid form. "Crystal needles" and sphaerocrystals are ordinary reserve material. These crystalloids are always found in the cytoplasm and only in algae having no pyrenoids in their chromatophores. They have been reported by many workers in the Siphonales and Siphonocladales.

In nearly related genera belonging to the Phyllosiphonaceae both oil and starch appear. In other families, *Udotea*, according to Küster (23), and *Derbesia* and *Dichotomosiphon*, according to Ernst (12), produce more or less abundant starch, which is easily

demonstrable. OLTMANNS (33) would be inclined to call this starch the "earliest visible assimilation product."

SIPHONOCLADALES

All the marine algae belonging to this order manufacture starch.

ULVALES

The common green seaweeds, *Ulva*, *Enteromorpha*, and *Monostroma*, and related genera, all produce starch as a food reserve.

CHLOROCOCCALES

Starch production characterizes these forms, which for the most part live in fresh water. Oil, however, is found in certain unicellular green algae: in *Dunaliella* and *Haematococcus*, members of the Volvocaceae; and in *Chlorococcum* and *Cystococcus*, in the Chlorococcaceae. In the former two examples, SENN (39) believes that the oil may be considered reserve material; in the latter two genera, GERNECK (15) reports oil and starch occurring simultaneously. Here one may assume that the oil is formed from carbohydrates.

CONJUGALES

In the case of *Spirogyra* and of *Mesotaenium*, and other Desmidiaceae, the resting zygote is filled with a fatty oil. Many investigators have stated that starch, first present in the zygote, disappears and is replaced by oil. Later, at the time of germination, starch reappears. From this it may be concluded that different organs of the same species are capable of storing different materials.

ULOTRICHALES, CHAETOPHORALES, AND CHARALES

The oil possessed by algae belonging to the Trentepohlieae is believed by SENN (39) to be a reserve material. As a rule all the other members of these three orders produce starch by means of the chloroplastid.

Volutin is produced more or less abundantly in the Volvocaceae, *Tetraspora*, Desmidiaceae, Zygnemaceae, and *Coleochaete*. It may be regarded with certainty as a reserve material.

Mannite has not been observed in green algae.

In a considerable number of the Chlorophyceae the physiological

agreement with higher plants has been ascertained without difficulty, as indicated by the investigations of FAMINTZIN (13) and KRAUS (22). It was found that *Spirogyra* loses its starch in darkness, but that, immediately upon being placed again in the light, it begins to build new starch.

Just as there are starchy seeds and oily seeds in the flowering plants, so there are the same two types of reserve substance in the resting zygote of the green algae. From the green algae up through the flowering plants, therefore, true starch and oil remain as characteristic reserve foods. Pentosan also occurs, but as a secondary reserve of far less importance.

In briefly reviewing the history of pigmentation in the algae, it will be noted that from the beginning there have been present a "chlorophyll-pair," possibly representing the structure elaborated as the food-making constituent of the pigment group, and a "carotinoid-pair," possibly having some auxiliary function, as that of removing the oxygen from chlorophyll *b*, as suggested by WILLSTÄTTER and STOLL. It may be that the pigments phycocyanin, phycoerythrin, and fucoxanthin, each in its turn, had the function of absorbing available rays of light and passing them on to the food-manufacturing group. At the present time almost nothing is known about the pigments and their functions in the algal groups, especially in the Cyanophyceae, Rhodophyceae, and Chrysophyceae. It must be noted, however, that the so-called "chlorophyll-carotinoid" combination of the higher plants had its origin in the algae of the Cyanophycean period, became partly or wholly modified in the Rhodophyceae, and at least appeared in the Phaeophyceae, as the complex structure that has continued apparently practically unchanged throughout all the succeeding geological ages.

A list of reserve materials reported by various authorities is appended:

Cyanophyceae:	Glycogen
Rhodophyceae:	Floridean starch (KYLIN)
Phaeophyceae:	Fucosan (KYLIN)
Chrysophyceae:	Oil (KLEBS, BOHLIN, BEIJERINCK)
	Leucosin (CZAPEK, PASCHER, WEST and FRITSCH)
	Paramylon (GOTTLIEB)

Chlorophyceae: Oil: in some of the marine forms and in resting zygotes (BORODIN)

Starch: for the first time in the history of plant life, true starch was formed as the product of metabolic activity

Summary

Inasmuch as the newer technique in spectral analysis and microchemistry has not yet been extensively applied to studies of pigments and food reserves in the various algal groups, one hesitates to make categorical statements regarding these matters at the present time. While a great amount of work has been done in the past, it has been carried on for the most part by individuals interested in but a small group of algae, or in but a limited phase of the subject. There is need for comparative chemical and physiological work embracing the pigments and products of assimilation in all of the classes of algae, as well as in the higher plants.

In the absence of precise knowledge it may be stated that, in the passing from one algal group to the next (plate I), with every change in the pigment content of the chromatophore there apparently has been a corresponding change in the "metabolic level" of the whole organism. Possibly this change in the chromatophore—morphological perhaps, physiological certainly—has directly or indirectly influenced the type of reserve food products that the organism is able to synthesize.

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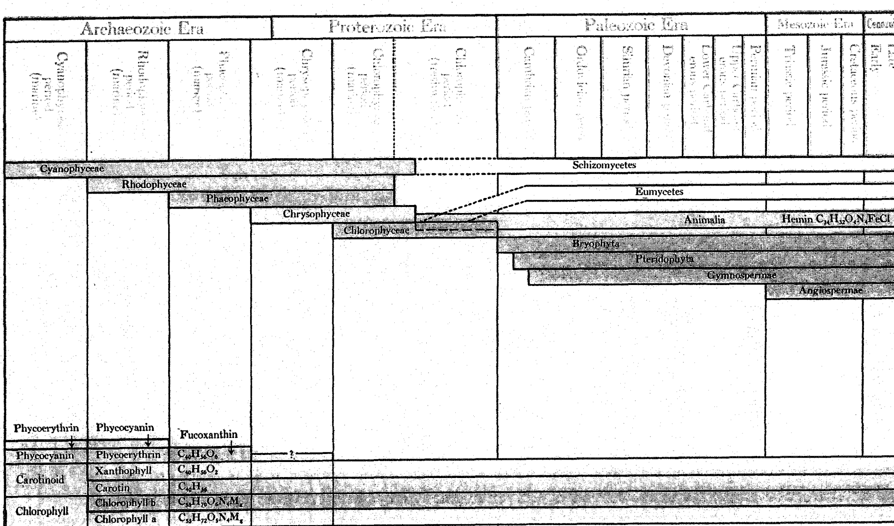
Archaeozoic Era
Proterozoic Era
Paleozoic Era
Mesozoic Era
Cenozoic
Cyanophyceae period (marine)
Rhodophyceae period (marine)
Triassic period
Jurassic period
Cretaceous period
Late
Early
Cyanophyceae
Rhodophyceae
Phaeophyceae
Chrysophyceae
Chlorophyceae
Schizomycetes
Eumycetes
Animalia
Hemin $C_{34}H_{32}O_4N_4FeCl$
Bryophyta
Pteridophyta
Gymnospermae
Angiospermae
Phycoerythrin
Phycocyanin
Fucoxanthin
Phycocyanin
Phycoerythrin
$C_{40}H_{56}O_6$
Carotinoid
Xanthophyll
$C_{40}H_{56}O_2$
Carotin
$C_{40}H_{56}$
Chlorophyll
Chlorophyll b
$C_{55}H_{70}O_6N_4M_g$
Chlorophyll a
$C_{55}H_{72}O_5N_4M_g$
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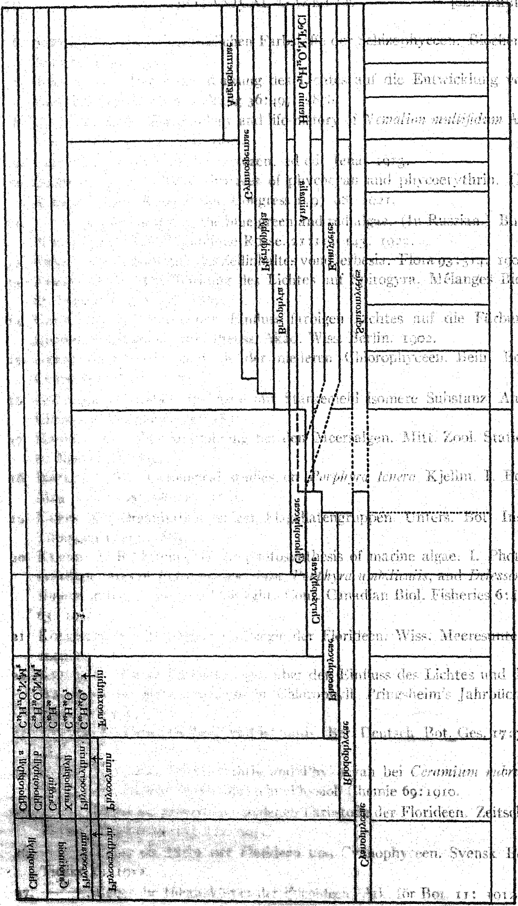

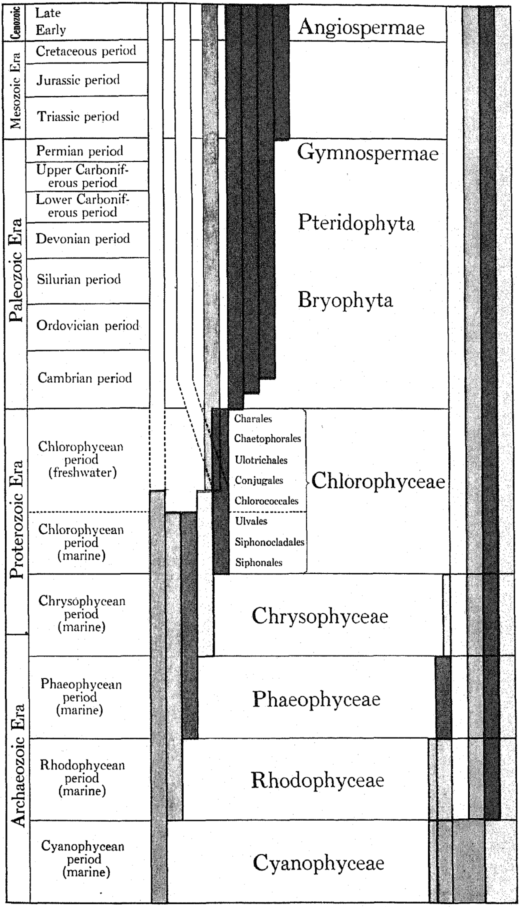

A GEOLOGICAL TIME TABLE INCLUDING THE FIVE ALGAL PERIODS

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NEW OR OTHERWISE NOTEWORTHY COMPOSITAE. IX

EARL EDWARD SHERFF

Raillardia demissifolia sp. nov.—Fruticosa forsitan arborescens, ramis pubescentibus conferte usque ad inflorescentiam foliosis. Folia opposita, sessilia, sicca plana vel interdum convoluta, 3-nervia nervis supra impressis, valde patentia vel mox demissa, lanceolato-oblonga, coriacea, faciebus glabrata vel infra sparsissime adpresso-hispida, supra subnitida, marginibus integra vel scabrido-ciliata, apice subobtusa, circ. 1.8–2.7 cm. longa et 5–9 mm. lata. Capitula laxe in panicula ∓1 dm. longa disposita, subnumerosa, pedicellata pedicellis gracilibus glanduloso-hispidis ultimis 0.5–2 cm. longis, ∓8-flora. Involucrum non visum. Achaenia lineari-clavata, atra, adpresse erecto-hispida, corpore 4–5.3 mm. longa.

Specimens examined: *Joseph F. Rock*, alt. 6000 ft., Haleakala, Isl. Maui, Hawaiian Isls., October, 1910 (type in Herb. Gray).

Raillardia reticulata sp. nov.—Fruticosa vel arborescens, demum 6–7.5 m. alta, valde ramosa ramis purpurascentibus pubescentibusque. Folia opposita, patentia, sessilia, plana vel marginaliter raro subrevoluta, oblonge lanceolata vel lineari-spathulata, basi vix subamplexicaulia apice acuta, coriacea, supra plus minusve subnitida, utrinque adpresse albido-hispida, marginibus ciliata et minutissime paucidenticulata, longitudinaliter circ. 5–7-nervia sed inter nervos plerumque reticulata, 3.5–5.5 cm. longa et nunc subangusta 5–7 nunc latiora 7–13 mm. lata. Capitula paniculata vel rarius paniculato-subcorymbose disposita, saepe numerosissima, conferta, ∓6-flora, pedicellata pedicellis tenuibus glanduloso-hispidis saepius 2–4 mm. longis. Involucrum anguste cylindraceum valde glandulosum sparsim villosum circ. 6–7 mm. longum, bracteis 4–8 non demum separatis. Achaenia nigra, tenuiter lineari-obconica, suberecte albido-hispida vel infra glabrata, circ. 4.5 mm. longa.

Specimens examined: *Joseph F. Rock* 8573, shrub, Puunianiau Crater, Haleakala, Isl. Maui, Hawaiian Isls., October, 1910 (2 type sheets in Herb. Gray); *idem* 8590, a tree 20–25 ft. high, alt. 6000 ft.,

above Ukulele, Haleakala, Isl. Maui, Oct. 11, 1910 (Herb. Gray, 3 sheets).

RAILLARDIA MONTANA **longifolia** var. nov.—Rami brunneo-subpurpurascentes. Folia opposita, patentia, subrhomboide elliptico-lanceolata, aegre coriacea, non nitida, glabrata, 5- vel 7-nervia, 5–6.5 cm. longa. Capitula circ. 8–10-flora. Achaenia corpore 4.5–5.5 mm. longa.

Specimens examined: *Joseph F. Rock* 8603, Kaupo Gap, Haleakala, Isl. Maui, Hawaiian Isls., Oct. 22, 1910 (type in Herb. Gray).

RAILLARDIA PLATYPHYLLA **leptophylla** var. nov.—Folia confertissima, anguste lanceolata, plerumque 6–7 cm. longa et 1–1.5 cm. lata.

Specimens examined: *Joseph F. Rock* 8578, on Puunianiau Crater, Haleakala, Isl. Maui, Hawaiian Isls. (type in Herb. Gray).

RAILLARDIA MENZIESII **angustifolia** var. nov.—Folia opposita, linearia vel anguste oblongo-elliptica, 3–5.5 cm. longa et 5–12 mm. lata.

Specimens examined: *Joseph F. Rock* 8546, alt. 6000 ft., Haleakala, Isl. Maui, Hawaiian Isls., September, 1910 (type in Herb. Gray); *idem* 8579, summit of Haleakala, October, 1910 (Herb. Gray).

Raillardia rocki sp. nov.—Fruticosa, ramis pilosis, purpurascentibus, moderate foliosis. Folia alterna, plana vel subplana, spathulato-oblonga vel superiora oblongo-lanceolata, coriacea et supra nitida, inferne ad basim sessilem saepius sensim angustata, apice subobtusa, marginibus hispidulo-ciliata et ∓3-denticulata, faciebus glabra vel obsolete adpresso-hispida, manifeste 3-nervia nervis supra impressis, 3.5–4.5 cm. longa et 7–9.5 mm. lata. Capitula (∓45 pro unica inflorescentia) paniculate vel subracemose disposita inflorescentia ∓1.9 dm. longa ramis gracilibus villosis bracteatis (bracteis magnis et foliis caulis similibus), ∓14-flora, corollis sulphureo-flavis pappo paululum brevioribus. Involucrum subanguste campanulatum, viride vel demum purpurascens, hispidulum, bracteis 7–9, connatis vel demum interdum pro parte parva separatis. Achaenia nigra, lineari-clavata, basi manifeste angustata, hispida vel inferne glabrata, 4–5 mm. longa.

Specimens examined: *Joseph F. Rock* 8601, Laie, Kaupo Gap, Haleakala, Isl. Maui, Hawaiian Isls., Oct. 22, 1910 (type in Herb. Gray).

Raillardia coriacea sp. nov.—Fruticosa, caule forsitan decumbens saltem ramis confertissime foliosis ∓4 dm. altis erecta. Folia sessilia, plus minusve terno-verticillata sed superiora alterna, oblongo-linearia, valde reflexa vel superiora adscendentia, coriacea, faciebus glaberrima, supra nitidissima, 3-nervia nervis supra impressis, marginibus revolutis sparsim spinuloso-ciliata et apicem versus 1–4-denticulata, subplana vel longitudinaliter convoluta, 4–5.5 cm. longa et pansa circ. 5–6.5 mm. lata. Capitula numerosa (∓60–80 pro unica inflorescentia), in panicula ∓2 dm. longa disposita, ∓10-flora, pedicellis ultimis tenuibus saepius 3–7 mm. longis, corollis flavis pappo vix aequantibus. Involucrum cylindraceo-campanulatum, hispidulum, purpurascens, 5–6.5 mm. longum, bracteis 6–9 demum plus minusve separatis. Achaenia nigra, oblanceolata, glabra, tantum circ. 3.8–4.2 mm. longa.

Specimens examined: *Joseph F. Rock* 8638, Koolau Gap, Haleakala, Isl. Maui, October, 1910 (type in Herb. Gray).

DUBAUTIA LAXA **obovata** var. nov.—Folia obovata, adpresso-hispida, circ. 6–7 cm. longa.

Specimens examined: *A. A. Heller* 2902 *pro parte*, Isl. Oahu, Hawaiian Isls. (type in Herb. Kew).

More than twenty years ago, in the course of a monographic study of the genus *Bidens*, my attention was called to the genus *Lipochaeta*, all the recognized species of which are native to the islands of the Pacific Ocean, and certain species of which (for example, *L. micrantha* A. Gray and *L. tenuifolia* A. Gray) through their foliage offer a deceptive resemblance to some species of *Bidens*. Subsequently, Mr. C. N. FORBES, of the Bishop Museum in Honolulu, secured for me a large assemblage of Hawaiian specimens of *Lipochaeta* and most of these were deposited in the Herbarium of Field Museum of Natural History, Chicago, for future study. Since then certain matters concerning *Lipochaeta* have received treatment in my writings. In the main, however, work on the genus was deferred until the summer of 1932. At that time I was urged by Professor OTTO DEGENER to make at once a complete monographic study of the genus, to the end that he might utilize the treatment for his large and monumental Flora Hawaiiensis, now appearing in frequent in-

stallments. A revisional study was at once begun. Professor DEGENER very generously placed at my disposal his many field notes, unpublished portions of manuscript, and a vast number of *exsiccatae*. These last have been determined and labeled, and, whenever the quantities permitted, distributed through Field Museum to the principal herbaria of the world. In all cases where DEGENER has anticipated my conclusions or has been the discoverer of novelties, he has kindly permitted the use of his name in joint authorship. This procedure has been followed, therefore, except in the case of *L. degeneri*, which has been named in his honor (despite his modest protest). Several of the forms treated have been delineated in excellent full-page plates by some of his associated artists for the Flora Hawaiiensis, and various others will likewise be illustrated in the near future.

Most of the world's principal herbaria have lent their entire *Lipochaeta* collections for this research. As a result, every type specimen of real consequence has been made accessible, also many cotypes (the word cotypes being used consistently throughout my writings to connote duplicates of the type, *ex numero et caeteris*). A key to all the species, a list of species excluded, etc. must be deferred, for lack of space, to a subsequent paper in which an extended monographic treatment of the genus will be presented.

Lipochaeta alata sp. nov.; *L. calycosa* var. *β*. Hillebr. Fl. Haw. Isls. 207. 1888.—Fruticosa, erecta, circ. 6 dm. alta, ramosa, ramis plus minusve angulatis primum dense scabrido-hispidis demum subglabratis. Folia opposita infra in petiolos breves (4–15 mm. longos) plus minusve alato-marginatos basi saepe aegre connatos contracta, petiolo adjecto 5–9 cm. longa et 3.5–5.5 cm. lata, lamina indivisa deltoideo-ovata vel rhombeo-ovata membranacea (demum saepe perspicue venosa) supra viridia scabridaque subtus pallidiora canescenti-hispidaque, apice acuta vel breviter acuminata, margine acriter duplicato-serrata. Capitula cymose disposita, tenuiter pedicellata pedicellis nunc hispidis nunc glabratis saepius 5–10 mm. longis, radiata, pansa ad anthesin 1.5–2 cm. lata et circ. 7–8 mm. alta. Involucri hispidi bracteae exteriores 4 vel 5 oblongae vel anguste ovatae 2–3.2 mm. longae, interiores latiores saepe paulo longiores, apice angustatae. Flores ligulati plerumque 7–10 interdum 10–14, flavidi, ligula lineares vel elliptico-oblanceolati, apice 2– (vel inter-

dum sub-3-) lobulati, circ. 7-9 mm. longi. Achaenia oblanceolata vel obovata, plus minusve triquetra, faciebus inferne glabrata superne verrucosa hispidulaque, nigra, angulis phellodonta, corpore 2.5-3.2 mm. longa, apice plerumque 2- (saepe imperfecte 3-) aristata aristis 0.2-0.5 mm. longis, inter aristas squamellis erectis coronata.

Specimens examined: *Abbé Urbain Faurie* 1007, Waimea, Isl. Kauai, March, 1910 (Herb. Deless., 2 sheets); *A. A. Heller* 2563, along the Hanapepe River, near the Falls, Isl. Kauai, Hawaiian Isls., Jul. 12, 1895 (type, Herb. Field Mus.: cotypes, Herb. Bish. Mus.; Herb. Gray; Herb. N.Y. Bot. Gard.; Herb. Par.; Herb. U.S. Nat.); *idem* 2563*a*, in a thicket, Hanapepe Valley, some three miles above station for no. 2563, *eodem tempore* (Herb. Deless.; Herb. Field Mus.); *H. Mann* and *W. T. Brigham* 542, alt. 2000-3000 feet, Waimea, Isl. Kauai (Herb. Field Mus.; Herb. Gray).

LIPOCHAETA ALATA **acrior** var. nov.—A specie foliis acerrime laciniato-dentatis apice perspicue acuminatis differt.

Specimens examined: *U.S. Exploring Expedition under Capt. Wilkes*, Isl. Kauai, Hawaiian Isls., 1840 (type, Herb. U.S. Nat.: cotype, Herb. Gray).

LIPOCHAETA sect. **Macraea** (Hook. *f.*) comb. nov.; *Macraea pro genere* Hook. *f.*, Proc. Linn. Soc. **1**:278. 1845; *etiam* Trans. Linn. Soc. **20**:209. 1847; *Trigonopterum* Steetz *in* Anders. Enum. Pl. Galap., Trans. Acad. Sc. Stockh. **1853**:161. 1853.—BENTHAM & HOOKER (Gen. Plant. **2**:373. 1873) recognized two sections in the genus *Lipochaeta*, namely, *Microchaeta*[1] (*pappi aristis prominulis*) and *Aphanopappus* (*pappo ad coronulam minutam reducto vel omnino deficiente*). To the latter they referred *Macraea* Hook. *f.*, a genus founded upon a highly anomalous Galapagos Island species, *L. laricifolia* (Hook. *f.*) A. Gray.[2] A study of all the known species of *Lipochaeta*, however, shows that their differentiation in nature has evolved more in respect to foliage and to grosser characters of capitula than in respect to achenes. Proper recognition of this fact compels placing the anomalous *L. laricifolia* apart from each of the

[1] Spelled by them *Microchaete*, but clearly a mere reduction from generic rank of NUTTALL'S *Microchaeta* (Trans. Amer. Philos. Soc. ser. II, **7**:450. 1841).

[2] All other species of *Lipochaeta* as recognized since the revision by ASA GRAY (Proc. Amer. Acad. **5**:129-131. 1861) are native to the Hawaiian Islands except *L. lifuana* Hochr., of the Loyalty and New Hebrides Islands.

two sections maintained by BENTHAM & HOOKER and according to it a separate sectional classification.

Lipochaeta acris sp. nov.—Fruticosa, erecta, ramosa, scabrida setis brevibus adpressis. Folia opposita breviter petiolata petiolis vix marginatis 0.5–1 cm. longis, petiolo adjecto 8–12 cm. longa et 3–7 cm. lata, simplicia, ovata vel anguste rhombeo-ovata, membranacea, basi late vel sublate cuneata, apice moderate vel longe acuminata, marginibus acriter perspicueque duplicato- vel laciniato-dentata dentibus 4–7 mm. longis apice subulatis. Capitula cymose disposita, tenuiter pedicellata pedicellis saepius 1–3 cm. longis, radiata, pansa ad anthesin 2.2–2.5 cm. lata et circ. 7–8 mm. alta. Involucri hispiduli bracteae exteriores circ. 5, oblongo-lineares vel ovatae, superne saepe angustatae vel acuminatae 3–5 (rarius –7) mm. longae, quam interiores plerumque paulo longiores. Flores ligulati 10–14, flavidi, ligula oblongi vel elliptico-oblanceolati, apice minute circ. 3-denticulati, 7–12 mm. longi. Achaenia cuneato-obovata, atra, plus minusve triquetra, faciebus glabra nitidaque, duobus marginibus plus minusve spinulosa, corpore circ. 2.5 mm. longa et circ. 1.5 mm. lata, apice truncato breviter biaristata et minute setulosa.

Specimens examined: *H. Mann* and *W. T. Brigham* 540, alt. 2000–3000 feet, Waimea, Isl. Kauai (type, Herb. Field Mus.: cotypes, Herb. Deless., 2 sheets; Herb. Gray; Herb. Mo. Bot. Gard.).

Lipochaeta forbesii sp. nov.—Fruticosa, erecta, omnino brevissime sed dense adpresso-hispidula, ramosa ramis subteretibus griseo-brunneis. Folia petiolata petiolis laciniate plus minusve alato-marginatis circ. 1.5–2 cm. longis, petiolo adjecto 5–9 cm. longa, plerumque incise 3–5-lobata, membranacea, lobis acriter vel etiam laciniate dentatis apice acutis vel acuminatis terminali multo majore rhombeo-ovato ad oblongo-lanceolato. Capitula numerosa cymose disposita, pedicellata pedicellis plerumque 0.5–2.5 cm. longis, radiata, pansa ad anthesin $\mp$ 2 cm. lata et circ. 5–6 mm. alta. Involucri hispidi bracteae exteriores 4–6, oblongo-lanceolatae vel lineari-oblongae, apice plus minusve acutae, 2–3 (raro –3.5) mm. longae. Achaenia polymorpha, atra, exteriora moderate vel late cuneata, 3–4-gona, angulis interrupte alata alarum segmentis saepe erecte interneque hamosis vel rostratis, faciebus praecipue superne spinu-

loso-verrucosa, corpore 2.5–3 mm. longa, apice breviter pauciterque squamelliformi-aristata.

Specimens examined: *C. N. Forbes* 1916*M*, Nuu, south slope of Haleakala, Isl. Maui, Hawaiian Isls., Mar. 9, 1920 (type, Herb. Field Mus.).

Named for the late Mr. FORBES who, during the last few years preceding his death, sent many specimens of *Lipochaeta* and *Bidens* to the Herbarium of Field Museum of Natural History as material for this research. As to foliage, the habit suggests that of *L. rockii* var. *subovata* (*vide* p. 101), of the Island of Molokai, but in the abundance and size of capitula the resemblance to *L. kahoolawensis* (*vide* p. 98), of the Island of Kahoolawe, is closer.

Lipochaeta degeneri sp. nov.—Fruticosa, plus minusve erecta, multum ramosa ramis virgatis griseis vel rubido-griseis teretibus subcanescentibus (setis albidis valde adpressis), saltem 3–4 dm. alta. Folia vix petiolata petiolis ∓1.5 mm. longis, spathulata vel lineari-oblanceolata, apice obtusa vel rotundata, membranacea, viridia, subrevoluta, integra vel obscure denticulata, 0.7–3 cm. longa et 2.5–5.5 mm. lata. Capitula numerosa tenuiter pedunculata pedunculis solitariis vel 3–5-adgregatis 1–3 cm. longis, radiata, pansa ad anthesin ∓1 cm. lata et circ. 5 mm. alta. Involucri hispidi bracteae subaequales, exteriores circ. 4, ovatae, obtusae, circ. 2 mm. longae. Flores ligulati 4–6, subflavidi, ligula oblongi, apice plerumque tridenticulati, circ. 5–6 mm. longi. Achaenia atra, exteriora cuneata, triquetra, angulis suberoso-alata, faciebus muriculata, corpore 1.5–2 mm. alta, apice erecte 2-3-aristulata et plus minusve setosa; interiora obcompressa, anguste cuneata, exalata, faciebus inferne glabra superne erecto-setulosa, corpore circ. 2 mm. longa, apice truncato erecte fimbriata et plerumque biaristulata.

Specimens examined: *Otto Degener* 4198, hot, arid, boulder-covered plain near sea, near southwest point of Isl. Molokai, May 16, 1928 (type, Herb. Field Mus.); *C. N. Forbes* 59*Mo*, Kalaeokalaau, Isl. Molokai, June, 1912 (Herb. Field Mus.); *J. F. Rock* 10288, *eodem loco*, 1910 (Herb. Gray).

LIPOCHAETA INTEGRIFOLIA **argentea** var. nov.—A specie foliis minoribus (plerumque 6–14 mm. longis et 1–4 mm. latis) saepius oblongo-linearibus perspicue canescentibus vel argenteis, ramulis plerumque canescentibus differt.

Specimens examined: *Abbé U. Faurie* 944, Wailuku, Isl. Maui, August, 1909 (Herb. Brit. Mus.); *Wm. Hillebrand*, above Maalae Bay, Isl. Maui (Herb. Berl.); *A. S. Hitchcock* 15131, sandy beach, western part of Isl. Molokai, Oct. 12, 1916 (U.S.); *Mann* and *Brigham* 371, on sandy isthmus, Isl. Maui (type, Herb. Field Mus.: cotypes, Herb. Deless.; Herb. Field Mus.; Herb. Gray; Herb. U.S. Nat.); *U.S. Exploring Exped. under Capt. Wilkes*, sand hills, Isl. Maui, 1840 (Herb. N.Y. Bot. Gard.; Herb. U.S. Nat.).

Lipochaeta integrifolia (Nutt.) A. Gray, of which I have had opportunity to examine numerous specimens, is distributed over the Islands of Molokai, Oahu, Kauai, Laysan, and Kure of the Hawaiian Archipelago (including the Leeward Islands).[3] The var. *argentea*, which for many years has been confused with the species proper, is known to me only from the Islands of Maui and Molokai.

Lipochaeta integrifolia **gracilis** var. nov.—Habitu gracilis, ramis (siccis) brunneo-atris elongatis tenuissimis, foliis moderate canescentibus linearibus tantum 7–12 (raro –16) mm. longis et 1–2 mm. latis, basi saepe in petiolum parvum angustata, nonnullis principalibus sub medium utrinque 1-dentatis vel parce 1-lobulatis.

Specimens examined: *Charles Gaudichaud* (*Freycinet's Voyage*) 217, Hawaiian Isls., September-October, 1836 (type, Herb. Gray: cotype, Herb. Par.); *idem, sine num., eodem loco* (Herb. Berl.; Herb. Par.).

Easily recognized by its slender habit, narrowly linear leaves, and by the fact that these are often provided with a conspicuous tooth or even small lobe on each lateral margin.

Lipochaeta integrifolia **major** var. nov.—Dense adpresso-hispidula sed non perspicue argentea, ramis (ramulis) saepe 1.2 dm. longis, foliis usque ad 4 cm. longis et 1 cm. latis, spathulatis, saepe crenatulatis, apice saepius obtusissimis, basi saepe breviter petiolatis petiolis usque ad circ. 5 mm. longis.

Specimens examined: *Guppy*, rocky sea-coast, Isl. Oahu, 1897 (Herb. Kew); *A. A. Heller* 2092, on the old lava flow back of Diamond Head, Isl. Oahu, Apr. 8, 1895 (type, Herb. Gray: cotypes, Herb. Deless.; Herb. Field Mus.; Herb. Mo. Bot. Gard.; Herb. N.Y. Bot. Gard.; Herb. Par.; Herb. U.S. Nat.).

[3] Laysan and Kure are here cited on the authority of Christophersen & Caum, Vascular Pl. Leeward Isls., Bishop Mus. Bull. 81. 39. 1931.

A much coarser plant than the species proper. Not to be confused with the unnamed var. β of HILLEBRAND'S Flora of the Hawaiian Islands (p. 208. 1888), the type of which was collected at Kailua of the same Island and is still extant (Herb. Berl.). This latter is found to be merely a scarcely distinguishable form of the species itself.[4]

LIPOCHAETA INTEGRIFOLIA **megacephala** Degener & Sherff var. nov.—Habitu var. *majori* similis sed differt foliis interdum longe petiolatis (usque ad 1.5 cm.), capitulis pansa ad anthesin 1.5–2 cm. latis.

Specimens examined: *E. Christophersen* 1400, in sand at alt. ∓5 m., Kaena Point, Isl. Oahu, Dec. 14, 1930 (type, Herb. Field Mus.); *Otto Degener*, *K. K. Park*, and *W. Hirai* (*Degener* distrib. no.) 4179, on arid clay soil near the ocean, Kaena Point, Isl. Oahu, Mar. 21, 1931 (Herb. Field Mus.); *Guppy*, Kaena Point, Isl. Oahu, 1897 (Herb. Kew).

This and the foregoing varieties may be separated from the species itself according to the following key:

a. Folia 1–2 mm. lata, nonnulla principalia sub medium utrinque 1-dentata vel parce 1-lobulata....................var. *gracilis*
a. Folia plerumque latiora (nisi pro var. *argentea*), principalia integra vel obscure denticulata
  b. Folia usque ad 4 cm. longa et 1 cm. lata
    c. Capitula pansa ad anthesin circ. 1.2 cm. lata....var. *major*
    c. Capitula pansa ad anthesin 1.5–2 cm. lata...var. *megacephala*
  b. Folia plerumque 0.6–2.5 cm. longa et 1–7 (raro –9) mm. lata
    c. Folia plerumque 1–2.5 cm. longa et 3–7 (raro –9) mm. lata *L. integrifolia* sensu stricto
    c. Folia plerumque 6–14 mm. longa et 1–4 mm. lata var. *argentea*

LIPOCHAETA SUCCULENTA var. **decurrens** (A. Gray) comb. nov.; *L. lanceolata* Nutt., Trans. Amer. Phil. Soc., ser. II, **7**:451. 1841; *L. australis* var. *decurrens* A. Gray, Proc. Amer. Acad. **5**:129. 1861; *L. connata* var. *decurrens* (A. Gr.) Hillebr. Fl. Haw. Isls. 206. 1888; *L. connata* var. *littoralis* Hillebr. *loc. cit.; L. variolosa* Lévl., Fedde Repert. **10**:122. 1911.

[4] As is also an additional specimen by *C. N. Forbes*, no. 615*Mo*, from Papoia, a small island near Kailua, April, 1914 (Herb. Field Mus.).

NUTTALL (*loc. cit.*) appears to have been the only observer to note the peculiar herbaceous habit of this plant: "Branches long and trailing, prostrate and ascending." The color, scabridity, and size of the leaves vary remarkably and, if it were not for the large assortment of interconnecting herbarium specimens available at present for study, one might well be misled into assuming that several distinct varieties were represented.

LIPOCHAETA SUCCULENTA **trifida** var. nov.—Varietati *decurrenti* similis sed foliis acriter trifidis vel trilobatis differt.

Specimens examined: *C. N. Forbes* 397*Mo*, Manawai, Isl. Molokai, Hawaiian Isls., August, 1912 (3 type sheets, Herb. Field Mus.).

LIPOCHAETA SUCCULENTA **barclayi** var. nov.—Fruticosa, saepe robusta, 9–12 dm. alta, caulibus siccis 4–6 mm. crassis. Folia nunc elliptico-oblonga nunc rhombeo-ovata, usque ad 1 dm. longa et 4.3 cm. lata, ad apicem mucronulatum plerumque rotundo-obtusa, margine leviter subremoteque denticulata dentulis subulatis vel mucronulatis, sicca subnitida et viridi-brunnea.

Specimens examined: *George Barclay* 1327, shrub 3 or 4 ft. high, in loam soil, hills, Isl. Atooi (Isl. Kauai), Hawaiian Isls., July, 1837 (type, Herb. Brit. Mus.); *C. N. Forbes* 71*K*, Kololau Trail, Isl. Kauai, Jul. 19, 1909 (Herb. Field Mus., 3 sheets).

LIPOCHAETA SUCCULENTA **angustata** var. nov.—Folia oblonge linearia vel lineari-lanceolata, principalia nunc 6–8 cm. longa et 3–5 mm. lata nunc paulo longiora et 8–12 mm. lata.

Specimens examined: *C. N. Forbes*, Isl. Kauai, 1909 (2 type sheets, Herb. Field Mus.).

The four varieties here given for *L. succulenta* (H. & A.) DC. may be separated from the species proper[5] according to the following key:

[5] The type of the species was collected, according to HOOKER & ARNOTT (Bot. Beechey's Voy. 87. 1832) on the Island of Oneeheow, now commonly known as Niihau. The type sheet (Herb. Kew) gives Oahu, however. Since the species is not otherwise known to me from Oahu, it may be that the plant actually did come from Niihau. Or possibly specimens were collected on both islands, the one from Oahu at first escaping the notice of investigators, while the one from Niihau failed to be preserved to the present day. Very probably HOOKER himself later saw the plant ascribed to Oahu, for its sheet bears the stamp of his private herbarium.

a. Folia oblonge linearia vel lineari-lanceolata. . . . . . . var. *angustata*
a. Folia latiora
  b. Folia plerumque trifida vel trilobata. . . . . var. *trifida*
  b. Folia indivisa
    c. Folia apice subacuta ad acuminata; plantis molokaiensibus var. *decurrens*
    c. Folia apice plerumque obtusa vel rotundata; plantis kauaiensibus niihauensibusque
      d. Folia rhomboide vel oblonge obovata vel oblanceolata *L. succulenta* sensu stricto
      d. Folia nunc elliptico-oblonga nunc rhombeo-ovata var. *barclayi*

LIPOCHAETA LAVARUM **ovata** var. nov.—Folia principalia ovata vel interdum subrhombeo-ovata, crenato-serrata, canescentia, apice obtusa, triplinervia nervis divergentibus, subtus venosissima, basi in petiolum saepius 1–1.5 cm. longum angustata, petiolo adjecto 4.5–6 cm. longa et 1.8–2.5 cm. lata.

Specimens examined: *C. N. Forbes* 2015*M*, Puu Onole, south side of Haleakala, Isl. Maui, Mar. 18, 1920 (Herb. Field Mus.); *J. F. Rock* 8674, below the crater at Kahikinui, Isl. Maui, November, 1910 (type, Herb. Gray: cotype, Herb. Field Mus.).

Forbes 2020*M*, from much the same vicinity in southeastern Maui (Kamana, southern slope of Haleakala), has foliage not really separable from that of *L. lavarum* proper. Thus *L. lavarum* is seen to be present in the vicinity whence came my type. Indeed the cited cotype sheet has, besides two sprays of the ovate-leaved form, one spray with numerous smaller and narrower leaves hardly atypic for *L. lavarum*. The ovate-leaved form, however, is known from nowhere else in the Hawaiian Islands and appears best construed as an endemic variety.

LIPOCHAETA LAVARUM **salicifolia** var. nov.; *L. lavarum* var. β, Hillebrand Fl. Haw. Isls. 207. 1888.—Folia secundaria oblongo-linearia, integra, valde membranacea, aegre venosa, supra viridia infra aegre pruinosa, 3.5–7.5 mm. lata.

Specimens examined: *E. Bishop*, near Lahaina, Isl. Maui, Hawaiian Isls. (type, Herb. Berl.); *C. N. Forbes* 2270*M*, Lahaina, Isl. Maui, May 8, 1920 (Herb. Field Mus.; *forma vix typica*).

HILLEBRAND'S type is a small flowering branch, less than 1.5 dm. long. This subdivides near the base into branchlets (besides a terminal peduncle), and the leaves present, although numerous, include none of the larger primary or principal leaves which on some specimens of *L. lavarum* occur only on the larger branches. Hence my leaf description must needs be regarded as incomplete. The leaves are thin or membranaceous and appear comparatively so green on the upper surface as to seem glabrous; but under a lens, abundant appressed, white hairs can be seen.

Forbes 2270*M* was collected in the type locality. The specimen cited is a handsome one, some 5 dm. long and with numerous leaves and various flowering and fruiting heads. It is probably to be construed as a slightly atypic form of this variety. The leaves are somewhat more veiny, at the apex more often acute, and the few principal leaves present measure up to 12 mm. in width. The larger the leaves the more tendency there is for them to become crenate-denticulate.

LIPOCHAETA LAVARUM **skottsbergii** var. nov.—Folia numerosa, strigoso-canescentia, linearia, 2–3.5 cm. longa et tantum 1.5–3 mm. lata.

Specimens examined: *Frederick Debell Bennett* 43, Isl. Maui, 1833–1836 (type, Herb. Berl.).

Through this variety *L. lavarum* is seen to approach, in foliage, the erect forms of *L. integrifolia.* The name is chosen in honor of C. J. F. SKOTTSBERG, Gothenburg, Sweden, who studied the type in 1925 and treated it as a narrow-leaved form of *L. lavarum.*

LIPOCHAETA LAVARUM **hillebrandiana** var. nov.; *L. lavarum* var. γ, Hillebrand Fl. Haw. Isls. 208. 1888.—Humilis, fruticosa, patens, divaricate ramosa. Folia secundaria lineari-oblonga vel spathulata, membranacea vel vix crassiuscula, sicca coriacea, triplinervia, canescentia, integra, apice obtusa vel quidem rotundato-obtusa, tantum 2–3 cm. longa et 3–7 mm. lata. Achaenia interrupte alata.

Specimens examined: *Wm. Hillebrand,* on rocks near sea, Lahaina, Isl. Maui (type, Herb. Berl.); *idem,* gulch back of Lahaina, August, 1870 (Herb. Berl., *cum specie ipsa commixta*); *Mann* and *Brigham* 374, mountain above Maalaea Bay, western Maui (Herb. Field Mus.; Herb. Gray); *Jules Remy* 277, Isl. Hawaii, 1851–1855 (Herb. Gray; Herb. Par.).

The HILLEBRAND herbarium (now incorporated in Herb. Berl.) has five specimens on the type sheet, four of them almost devoid of leaves. An additional sheet, however, bears two sprays of the species proper and one of this variety, all three coming from the type vicinity. HILLEBRAND himself appears to have overlooked this supplementary specimen of his variety. It bears among the numerous secondary leaves several of the larger primary or principal leaves, and these are 10–12 mm. wide but less than 5 cm. long. HILLEBRAND cited Maalaea Bay as a second locality for this variety. *Mann* and *Brigham* 374, from a mountain above Maalaea Bay, apparently has the more shrubby, dwarfed, and divaricately branching habit of the type material, but the leaves average nearly twice as long as in the type, and display in this respect an approach to those of the species proper.

LIPOCHAETA LAVARUM **longifolia** var. nov.—A specie internodiis forsitan elongatioribus foliis principalibus petiolo adjecto 7–13 cm. longis differt.

Specimens examined: *G. C. Munro* 202, Maunalei Valley, Isl. Lanai, Hawaiian Isls., Mar. 9, 1915 (type, Herb. Bish. Mus.).

L. lavarum (Gaud.)DC. and the foregoing varieties may be distinguished by the following key:

a. Folia principalia linearia, tantum 1.5–3 mm. lata
var. *skottsbergii*

a. Folia principalia latiora

  b. Folia principalia plus minusve ovata, 1.8–2.5 cm. lata
  var. *ovata*

  b. Folia principalia angustiora

    c. Folia secundaria numerosa, oblongo-linearia vel spathulata

      d. Folia secundaria 2–3 cm. longa. . . . . . .var. *hillebrandiana*

      d. Folia secundaria plerumque 4–7 cm. longa. . var. *salicifolia*

    c. Folia secundaria plerumque non numerosa, principalibus plus minusve lanceolatis similia

      d. Folia principalia petiolo adjecto 7–13 cm. longa
      var. *longifolia*

      d. Folia principalia petiolo adjecto 2.5–8 cm. longa
      L. lavarum sensu stricto

LIPOCHAETA SUBCORDATA **populifolia** var. nov.—A specie differt foliis moderate vel late ovato-cordatis vel ovato-subcordatis.

Specimens examined: *G. C. Munro* 670, Maunalei Valley, Isl. Lanai, June 18, 1918 (type, Herb. Field Mus.: cotype, Herb. U.S. Nat.).

LIPOCHAETA SUBCORDATA **membranacea** var. nov.—A specie foliis ovato-subcordatis vel subcordato-deltoideis, sparsim adpresso-hispidis itaque perspicue membranaceis utrinque viridibus.

Specimens examined: *E. Bishop* 14, back of Lahaina, western Maui (type, Herb. Berl.).

The type was originally in HILLEBRAND'S private herbarium and was the basis of his distributional note, "Maui! back of Lahaina (leaves deltoid, flower-heads larger)."

In *L. subcordata* A. Gray, the leaves tend to be narrowly deltoid, often suggesting in outline a moderately narrow isosceles triangle. The leaves are conspicuously and densely whitish-hispid beneath. The leaves of var. *populifolia* are broader in outline, much like those of *Populus deltoides* Marsh. The leaves of var. *membranacea*, of which I have seen only the small branch on the type sheet, are intermediate in outline but are noticeable for their sparse hispidity, this latter character being associated with more membranaceous texture and a closer similarity in color between the upper and lower surfaces. Much variation also occurs, within the species proper, as to size of leaves, length of internodes, and size of heads, but these variations appear in this species much too inconstant to serve as a basis for the delimiting of varieties.

LIPOCHAETA LOBATA var. **hastulata** (Hook. & Arn.) comb. nov.; *Verbesina hastulata* Hook. & Arn. Bot. Beechey Voy. 87. 1832; *L. hastulata* (Hook. & Arn.) DC. Prodr. 5:611. 1836.—ASA GRAY had labeled the type sheet, "*Lipochaeta australis* A. Gr. var. *lobata*." This is doubtless to be explained by the fact that he had already reduced *L. lobata* (Gaud.)DC. to a varietal rank and was disinclined to reduce the plant under observation to still lower rank, hence the equation of the two. It is doubtful, however, whether out in nature these merge.

The type (Herb. Kew) came from some locality on the Island of Oahu. With it must be associated a specimen collected by *Otto*

Degener, *K. K. Park*, and *W. Hirai*, no. 4181, among rocks, grass, and weeds on talus in dry region, along coast 2 miles east of Kaena Point toward Kawaihapai, Isl. Oahu, Mar. 22, 1931 (Herb. Field Mus.; *forma vix typica*).

LIPOCHAETA LOBATA var. **denticulata** (Wawra) comb. nov.; *L. calycosa* A. Gray, Proc. Amer. Acad. 5:130. 1861; *L. australis* var. *denticulata* Wawra, Flora **56**:77. 1873.

LIPOCHAETA LOBATA **leptophylla** Degener & Sherff, var. nov.—Folia lanceolata vel lanceolato-linearia, saepe attenuato-acuminata, usque ad 8 (raro 9) cm. longa. Involucri bracteae ovatae, apice plus minusve acuminatae.

Specimens examined: *C. N. Forbes* 2024O, at Kolekole Pass, Waianae Range, Isl. Oahu, Feb. 1–2, 1915 (3 type sheets, Herb. Field Mus.); *idem et Dean Lake* 2274O, talus slopes near Kaena Point, Isl. Oahu, Dec. 16, 1915 (Herb. Field Mus.); *C. N. Forbes* and *J. C. Bridwell* 2476O, ridge between Niu and Wailupe, Isl. Oahu, Apr. 11, 1917 (Herb. Bish. Mus.; Herb. Field Mus.); *J. F. Rock* 17122, Wailupe Valley, Isl. Oahu, Apr. 14, 1918 (Bish.).

LIPOCHAETA LOBATA var. **aprevalliana** (Dr.) comb. nov.—*L. aprevalliana* Drake del Cast. Illustr. Fl. Ins. Mar. Pacif. 71. *tab.* 34. 1886.

LIPOCHAETA LOBATA **albescens** var. nov.—Folia saepe 8–13 cm. longa, pallida, coriacea, supra subnitida et subglabrata, petiolis ac nervis principalibus etiam ramulis et pedicellis et involucri bracteis (late ovatis) demum stramineo-albis.

Specimens examined: *A. A. Heller* 2021, on steep slopes at Diamond Hill (Diamond Head), Isl. Oahu, Mar. 28, 1895 (type, Herb. Field Mus.: cotypes, Herb. Gray; Herb. N.Y. Bot. Gard.; Herb. U.S. Nat.).

LIPOCHAETA LOBATA **grossedentata** Degener & Sherff, var. nov.—Folia tenera, oblongo-lanceolata, vix petiolata petiolis circ. 2–3 mm. longis, principalia 6–8 cm. longa, marginibus grosse dentata, apice acuta. Capitula pansa ad anthesin circ. 2.5–2.9 cm. lata, ligulis pulchre aureis anguste obovatis circ. 5.5–7 mm. latis apice manifeste bilobulatis.

Specimens examined: *Otto Degener*, *K. K. Park*, and *Wm. Bush*, on rather dry, rocky, partly shaded slope in gulch north of middle

of ridge between Puu Pane and Puu Kamaohanui, Isl. Oahu, Hawaiian Isls., Jan. 10, 1932 (type, Herb. Field Mus., 2 sheets: cotype, Herb. Kew).

LIPOCHAETA LOBATA **hastulatoides** Degener & Sherff, var. nov.—Folia breviter alato-petiolata, ovata vel oblongo-ovata, subcoriacea, acriter serrata, apice saepius subacuta, omnino 3–6.5 cm. longa.

Specimens examined: *Otto Degener* 4305, Pohakea Gulch, south-easternmost part of West Maui, Hawaiian Isls., Jul. 11, 1927 (type, Herb. Field Mus., 2 sheets: cotypes, Herb. Berl.; Herb. Brit. Mus.; Herb. Gray; Herb. Kew; Herb. N.Y. Bot. Gard.).

Interesting as being the only form of *L. lobata* known from the Island of Maui. The leaf characters place the variety between var. *hastulata* and var. *denticulata*. The general aspect of the foliage is deceptively like that of some species of *Prunus* and *Pyrus* in the family Rosaceae.

LIPOCHAETA LOBATA **maunaloensis** var. nov.—Folia principalia 4–7 cm. longa, valde membranacea, manifeste hastato-trilobata lobis lateralibus late oblongis ad subrotundis, terminali elongato elliptico-oblongo.

Specimens examined: *C. N. Forbes 7Mo*, on Mauna Loa, Isl. Molokai (*nec alibi*), Hawaiian Isls., June, 1912 (type, Herb. Field Mus.).

The varieties of *L. lobata* may be severally distinguished from the species proper according to the following key:

a. Folia (raro 8–10 cm. longa) et ramuli et involucri bracteae virides vel subvirides
  b. Folia basi plus minusve cordata............var. *aprevalliana*
  b. Folia basi cuneata rotundatave
    c. Folia plus minusve hastulata
      d. Folia principalia saepe 10–12 cm. longa et 4–6 cm. lata; involucri bracteis exterioribus demum 5–7 mm. longis var. *hastulata*
      d. Folia principalia plerumque 3–6 interdum usque ad 8 (rarius 10) cm. longa
        e. Folia perspicue lobata, lobo principali anguste ovato vel rhombeo-ovato, planta molokaiensi var. *maunaloensis*

e. Folia aegre vel moderate lobata, lobo principali anguste deltoideo-oblongo, planta oahuensi
L. lobata sensu stricto

c. Folia non (vel rarissime sub-) hastulata

d. Omnia vel nonnulla folia ovata, involucri bracteis exterioribus oblongo-ovatis vel obovatis apice rotundo-obtusis vel subacutis

e. Petioli alati. . . . . var. *hastulatoides*

e. Petioli exalati. var. *denticulata*

d. Folia lanceolata vel lanceolato-linearia, serrata vel denticulata, involucri bracteis exterioribus ovatis apice plus minusve acuminatis. . . . . var. *leptophylla*

d. Folia oblongo-lanceolata, grosse dentata
var. *grossedentata*

a. Folia (saepe 8–13 cm. longa) pallida, ramulis involucri bracteisque albescentibus . . . . var. *albescens*

×**Lipochaeta procumbens** Degener & Sherff, hybr. nov.—Fruticosa, prostrata, ramosa, minute adpresso-hispidula, caulibus elongatis angulatis flexilibus, ∓8 dm. longis. Folia inferne in basim petioloideam cuneato-angustata, principalia omnino 3–7 cm. longa, rhombeo-oblonga, apice obtusa vel subacuta, sparsim subobsoleteque serrulata, crassiuscula, supra manifeste 3-nervia, infra elevato-venosa. Capitula tenuissime pedunculata pedunculis saepe 3–7 cm. longis, laxe disposita, radiata, pansa ad anthesin circ. 1.5 cm. lata et circ. 6 mm. alta. Involucri bracteae exteriores 4 vel 5, late ovatae ad apicem obtusae, 2–3.2 mm. longae (rarius 1 vel 2 minimae oblongae ∓1.5 mm. longae). Flores ligulati aurei 8–10, ligula anguste obovati, apice 2–3-denticulati, ∓7 mm. longi. Achaenia deficientia; ovariis sterilibus.

Specimens examined: *Otto Degener, K. K. Park*, and *W. Hirai* (*Degener* distrib. no.) 4178, forming mats on the loose lava rock on arid plain near ocean, Kaena Point, Isl. Oahu, Hawaiian Isls., Mar. 21, 1931 (3 type sheets, Herb. Field Mus.: cotypes, Herb. Kew; Herb. Munich); *Degener* 4303, *eodem loco*, Jan. 31, 1932 (Herb. Field Mus.).

The type specimens were growing among specimens of *L. lobata* var. *denticulata* and *L. integrifolia* var. *megacephala*, and were

suspected by DEGENER of being hybrids between those two varieties. His suspicion appears to be confirmed by the intermediate nature of the main diagnostic characters studied, also by the fact that the several hundred ripe capitula examined have invariably abortive ovaries, not a single achene having been matured.

Lipochaeta profusa sp. nov.—Fruticosa, erecta, omnino sed dense hispidula, ramosa ramis subteretibus. Folia opposita inferne abrupte in basim connatam acriter dentatam 8–12 mm. latam contracta, principalia circ. 7–9 cm. longa et 3.5–4.5 cm. lata, margine acriter sed irregulariter serrata, apice breviter acuminata, subtus perspicue elevato-venosa. Capitula cymose disposita, numerosa (unico ramo interdum quidem 50–75 capitula subtendente) pedicellis (ramulis ultimis) plerumque usque ad 2 cm. longis, radiata, pansa ad anthesin $\mp$15 mm. lata et 5–6 mm. alta. Involucri hispiduli minimi (demum tantum circ. 3 mm. alti et supra tantum circ. 3–3.5 mm. lati) bracteae exteriores 3 vel 4, ovatae, subacutae vel saepius abrupte acutae vel etiam breviter acuminatae, siccae apice plus minusve atrae, 2–3.2 mm. altae. Flores ligulati $\mp$8, flavi, ligula elliptico-oblanceolati, apice plus minusve bidenticulati, circ. 7–8 mm. longi. Achaenia exteriora brunneo-grisea vel atra, 3–4-gona, angulis interdum interrupte cartilagineo-alata, faciebus levia vel valde verrucosa, cuneata, corpore 2–2.2 mm. alta et supra 1–1.3 mm. lata, apice erecte spinuloso-setosa et erecte pauciaristata aristis brevibus et antrorsum hispidulis.

Specimens examined: *Mrs. Isabella Sinclair*, Isl. Hawaii, Hawaiian Isls., *commun.* January, 1885 (type, Herb. Kew).[6]

A species close to *L. alata* (*vide* p. 81), from which it differs in its leaf bases and inflorescence. *L. alata* has the leaves narrowed below into a manifestly winged petiole, while in *L. profusa* the leaf bases (which are distinctly connate), although amounting to a contraction of the blade, are about 8–12 mm. wide and would not be considered as being winged petioles. Furthermore, *L. profusa* has the inflorescence much more decompound and, in the species proper, the heads are comparatively minute.

[6] The type label gives "Hawaii," whence it is assumed that the island by that name. rather than the whole archipelago, later commonly known as Territory of Hawaii, was meant.

The type sheet bears the aboriginal name "Nehe." Elsewhere (Indig. Fl. Haw. Isls. pl. 21. 1885) Mrs. SINCLAIR illustrated a spray of Nehe, but her plate portrays an entirely different species (a form of *L. connata* Gaud. [DC.]).

LIPOCHAETA PROFUSA **robustior** Degener & Sherff var. nov.—A specie capitulis majoribus demum cum fructibus 6–8 mm. latis et 5–7 mm. altis differt.

Specimens examined: *Otto Swezey* (*Degener* distrib. no.) 4185, in lowlands, 2 miles from Kekaha, Isl. Kauai, Hawaiian Isls., Jul. 18, 1932 (type, Herb. Field Mus.).

LIPOCHAETA HETEROPHYLLA **molokaiensis** var. nov.—Forsitan robustior, foliis plerumque indivisis vel hastato-trilobis, siccis nunc caeruleo-viridibus nunc flavidulo-viridibus, lamina vel lobo principali rotundo ad lanceolato.

Specimens examined: *C. N. Forbes* 110*Mo*, Mauna Loa, western Molokai, June, 1912 (Herb. Field Mus., 2 sheets); *J. F. Rock* 10287, west end of Molokai, 1910 (type, Herb. Field Mus.: cotype, Herb. Gray; *pro specie nova a Rockio habita*).

A variety at first considered by me as a valid species and later found to have been previously so treated by ROCK, who appears from his several collections and his herbarium determinations to have given it special attention.

LIPOCHAETA HETEROPHYLLA **malvacea** Degener & Sherff, var. nov.—Fruticosa, valde divaricateque ramosa ramis ramulisque teretibus subcandidis scabrido-hispidulis vel demum nitido-subglabratis. Folia numerosa opposita alato-petiolata petiolis 5–15 mm. longis basi dilatato-connatis, petiolo adjecto 2–6 cm. longa, circumambitu ovata, plerumque 3-lobata lobis obtusis irregulariter dentatis vel quidem plus minusve sublobulatis utrinque scabrido-hispidis subtus perspicue venosis. Capitula numerosissima (unica herbarii scheda ∓100 ferente), cymose disposita pedicellis tenuibus 1–4 cm. longis, ad anthesin minima (pansa tantum circ. 6–7 mm. lata et circ. 3 mm. alta). Involucri scabrido-hispidi bracteae exteriores circ. 6, late oblongo-lanceolatae vel ovato-lanceolatae apice plus minusve acutae demum 3–4 mm. longae. Flores ligulati circ. 8, aurei, ligula obovati, apice acriter 2–3-denticulati, 3–4 mm. longi. Achaenia subbrunnea, valde verrucosa, obovato-cuneata, angulis

lateralibus alata alis saepe incisis vel interruptis apice in aristam squamelliformem incurvatam productis, corpore 2.4–3 mm. longa, exteriora superne 3–4-angulata angulis medianis plus minusve in aristam productis, interiora obcompressa omnia apicis centro plerumque tenuiter erecteque 2-aristata atque inter aristas erecte plurisetosa.

Specimens examined: *Otto Degener* 4199, on arid, rocky plain near Kolo, western Molokai, Hawaiian Isls., Apr. 5, 1928 (2 type sheets, Herb. Field Mus.: cotypes, Herb. Berl.; Herb. Brit. Mus.; Herb. Gray; Herb. Kew; Herb. Mo. Bot. Gard.; Herb. N.Y. Bot. Gard.; Herb. Par.).

This variety is so unique in appearance that for some time I regarded it as a distinct species. From *L. heterophylla* proper it is separated by its much more numerous heads, these being at anthesis only about 6–7 mm. wide, not 1–2.2 cm. wide as in *L. heterophylla.* The foliage when much dissected has a distinctly *malvaceous* aspect.

L. heterophylla and its two varieties may be identified by the following key:

Aegra, vix erecta, ramis gracilibus saepe subvolubilibus vel subscandentibus, foliis valde polymorphis, plantis lanaiensibus mauiensibusque raro molokaiensibus
L. heterophylla sensu stricto

Robusta, ramis validioribus nec subvolubilibus nec subscandentibus, foliis plerumque subsimplicibus vel hastato-trilobis siccis plerumque flavido-viridibus raro pedatim 3–5-lobatis, plantis molokaiensibus............................var. *molokaiensis*

Pro parte intermedia, plerumque ramosissima, foliis siccis caeruleo-viridibus saepius multo divisis, capitulis numerosissimis pansa ad anthesin plerumque sub 1 cm. latis, plantis molokaiensibus
var. *malvacea*

Lipochaeta bryanii sp. nov.—Suffruticosa, ramosa ramis angulatis vel etiam subalatis minute adpresso-hispidulis gracilibus. Folia numerosa, petiolata petiolis tenuibus usque ad 1.5 cm. longis, petiolo adjecto principalia circ. 4–5 cm. longa alia plerumque 2–3 cm. longa, circumambitu oblonga vel hastata, apice obtusa basi late cuneata margine leviter pauciterque crenato-dentata vel basi utrinque etiam 1-lobulata, rugulosa, densissime ac minutissime adpresso-

hispidula, subtus elevato-venosa. Capitula ad apices ramulorum disposita, tenuiter pedicellata pedicellis adpresso-hispidulis 1–4 cm. longis, radiata, pansa ad anthesin circ. 1.4–1.7 cm. lata et circ. 4–5 mm. alta. Involucri adpresso-hispiduli bracteae exteriores 4 vel 5, ovatae vel late oblongae, apice obtusae vel rotundatae, ad basim aegre gibbosae, 3.5–4.5 (raro –5) mm. longae. Flores pilosuli, ligulati plerumque 5, flavi, ligula oblongae vel anguste obovatae, apice bidenticulati, 6–8 mm. longi. Achaenia exteriora tumida orbiculata vel obovato-cuneata, 3–4-gona, valde verrucosa vel etiam verrucoso-spinulosa (praecipue ad apicis circumferentiam), angulis raro plus minusve interrupto-alata, corpore circ. 2–2.5 mm. alta, apice erecte hispida et ad centrum 2–3-aristata aristis subsquamelliformibus brevibus erectis acribus.

Specimens examined: *E. H. Bryan Jr.* 736, amid pili grass, on slope, Isl. Kahoolawe, Hawaiian Isls., Feb. 16, 1931 (type, Herb. Bish. Mus.).

The species is named in honor of the collector, *Edwin H. Bryan Jr.*, the Bernice Pauahi Bishop Museum in Honolulu. It was largely through his kindness that I was permitted to study this and other specimens of *Lipochaeta* belonging to that institution.

Lipochaeta kahoolawensis sp. nov.—Fruticosa, plus minusve ramosa ramis teretibus sulculatis adpresso-hispidulis. Folia alato-petiolata petiolis 1–2.2 cm. longis inferne late dilatato-connatis superne 2–6 mm. latis, petiolo adjecto 6–8 (raro –9) cm. longa, laminae circumambitu plus minusve deltoideo-ovata, palmatim 3–5-lobata lobis acutis vel subacutis, margine acriter irregulariterque serrata, supra adpresse scabrido-hispidula, subtus molliter ac dense pilosa. Capitula paniculato-cymose ad ramorum apices disposita (10–35-adgregata), tenuissime pedicellata pedicellis hispidis saepius 3–10 mm. longis, radiata, pansa ad anthesin 8–14 mm. lata et 4–5 mm. alta. Involucri hispidi parvi bracteae exteriores circ. 4, oblongo-lineares vel lanceolato-oblongae, apice subacutae, plerumque circ. 2 mm. longae. Flores ligulati 5–8, flavi, ligula anguste obovati, apice denticulati, circ. 6–7 mm. longi. Achaenia atra vel atro-brunnea, exteriora cuneata, 3–4-gona, faciebus minute verrucosa, angulis plus minusve (interdum interrupte) alata, corpore vix 2 mm. longa, superne plus minusve erecto-setosa, alarum apicibus in dentes erecto-

incurvatos tenues acres producta, suo apice centraliter saepius 2–3-aristata aristis brevibus.

Specimens examined: *Jules Remy* 269, Isl. Kohoolave (Kahoolawe), Hawaiian Isls., 1851–1855 (type, Herb. Par.).

The leaves suggest those (particularly the more definitely lobed ones) found on *L. heterophylla* var. *molokaiensis*. The inflorescence, however, is very distinct. This appears at first glance like a true panicle. The heads are numerous, minute, and in measurements for the exterior involucral bracts and exterior achenes may easily be distinguished from those of all related species.

Lipochaeta perdita sp. nov.—Suffruticosa, forsitan procumbens vel suberecta, ramosa ramis gracillimis rotundato-angulatis adpresso-hispidulis. Folia opposita petiolata petiolis tenuibus circ. 6–14 mm. longis, petiolo adjecto 2–4 cm. longa et 1–2.2 cm. lata, simplicia, ovato-deltoidea, apice subobtusa vel subacuta sed non acuminata, basi subtruncata vel truncata (nec vere subcordata), margine leviter sed subacriter crenato-serrata, supra subrigide subtus molliter adpresso-hispida. Capitula subsolitaria, longe tenuiterque pedunculata pedunculis adpresso-hispidis usque ad 9 cm. longis, demum (cum fructibus) circ. 12–13 mm. lata. Involucri adpresso-hispiduli bracteae exteriores 4 vel 5, ovatae vel subobovatae, apice subacutae vel subobtusae, circ. 5 mm. longae. Flores ligulati non visi. Achaenia exteriora obovato-cuneata, tumida, 3–4-gona, valde verrucosa, rubido-atra, angulis interdum plus minusve interrupto-alata, corpore 2.5–2.9 mm. longa et superne circ. 2.5 mm. crassa, apice ad circumferentiam sparsim erecto-spinulosa, aliter brevissime erecto-setosa et centraliter interdum pauciaristata aristis antrorsum hispidulis acribus.

Specimens examined: *David Nelson*, Hawaiian Isls., 1778–1779 (type, Herb. Brit. Mus.).

The general aspect is deceivingly like that of *L. subcordata* A. Gray. The leaves are smaller, however, than the average leaves of that species and are not so acuminate at the tips. The much larger capitula, these solitary or nearly so, are further means of distinction from *L. subcordata*. The type sheet bears five sprays, all closely similar. The species apparently has not been re-collected since the visit of NELSON to the Hawaiian Islands more than a century and a

half ago, and may well be one of those numerous endemic species which have been destroyed with the advent of civilization.[7]

Lipochaeta venosa sp. nov.—Subherbacea, ramosa ramis angulatis gracilibus scabrido-hispidis. Folia numerosa, tenuiter petiolata petiolis plerumque 4–10 mm. longis petiolo adjecto nunc omnia tantum 1–2 cm. longa nunc nonnulla 3–5 cm. longa, caeruleo-viridia, hastulato-trilobata, supra valde depresso- subtus valde elevato-venosa, supra sparsim breviterque hispidula subtus dense hispida lobis irregulariter crenato-dentatis. Capitula ad apicem ramulorum disposita, tenuiter pedicellata pedicellis villosis, radiata, pansa ad anthesin $\mp$1.3 cm. lata et circ. 5 mm. alta. Involucri villoso-hispidi bracteae exteriores circ. 5, oblongae vel late ovatae vel etiam perspicue dilatatae ac subrotundatae, apice obtusae, saepe patentes, 3–4.5 mm. longae. Flores ligulati circ. 6–8, flavi, ligula plus minusve oblongi, apice denticulati, $\mp$6 mm. longi. Achaenia matura minima, atra, cuneata vel obovata, inferne glabrata superne minute setulosa, angulis exalata vel superne vix cartilagineo-verrucosa, corpore 1.5–2.2 mm. longa, plerumque 5–6-aristata, aristis brevibus acribus stramineis antrorsum hispidulis.

Specimens examined: *J. F. Rock* 8349, Nohonaohae Crater, Waimea, Isl. Hawaii, June, 1910 (type, Herb. Field Mus.: cotype, Herb. Gray).

Lipochaeta rockii sp. nov.—Fruticosa, ramosa ramis teretibus vix sulculatis, hispidula. Folia tenuiter petiolata petiolis circ. 7–14 mm. longis, petiolo adjecto 3–5 cm. longa, circumambitu triangulato-ovata ad triangulato-cordata, 3–5-lobata vel subpedatim 3–5-partita, lobis membranaceis dentatis vel rursus lobulatis subrotundis ad lineari-oblongis plerumque obtusis terminali longiore vel etiam elongato ac lateraliter laciniato-dentato. Capitula subcorymbosa, tenuiter pedicellata pedicellis hispidulis plerumque 3–6 cm. longis, radiata, pansa ad anthesin 1.5–2.4 cm. lata et 5–6 mm. alta. Involucri hispiduli bracteae exteriores circ. 5 vel 6, nunc lanceolato-oblongae nunc ovatae vel obovatae sed ad apicem plus minusve acuminatae, 2–4 mm. longae. Flores ligulati circ. 8, flavidi, ligula

[7] The trivial name has been selected, however, with its twofold meaning in mind, namely, either *ruined* or *lost*.

moderate vel latissime oblongi, apice acriter bidentati, 7–9 mm. longi. Achaenia atra, faciebus minutissime adpresso-hispidula et saepe subverrucosa, exteriora 3–4-gona plus minusve alata alis interrupte erosis, omnia cuneata, corpore 2–2.8 mm. longa, apice truncato erecte setosa et ad centrum plerumque 2-aristata aristis tenuibus brevibusque.

Specimens examined: *Otto Degener* 4212 *pro minima parte*, dry, grassy, rocky plain near Waiahewahewa Gulch, Isl. Molokai, Apr. 18, 1928 (Herb. Field Mus.); *Abbé U. Faurie* 1001, Kamolo, Isl. Molokai, June, 1910 (Herb. Brit. Mus.; Herb. Deless., 2 sheets); *idem* 1002, *eodem loco et tempore* (Herb. Brit. Mus.; Herb. Deless.); *C. N. Forbes* 220*Mo*, mountains below Puu Kolekole, Isl. Molokai, July, 1912 (Herb. Field Mus., 3 sheets); *Wm. Hillebrand*, Isl. Oahu (Herb. Berl.; Herb. Kew); *idem*, Isl. Molokai (Herb. Berl., 2 sheets); *J. F. Rock* 6156 (type, Herb. Gray: cotype, Herb. Field Mus.).

This species and its varieties have commonly been confused with *L. lobata.*

LIPOCHAETA ROCKII **subovata** var. nov.—Folia principalia 4–7 cm. longa et usque ad 5 cm. lata, deltoideo-ovata vel subovata, grosse dentata vel inciso-lobulata, petiolis anguste alato-marginatis.

Specimens examined: *Jules Remy* 270, Isl. Molokai, 1851–1855 (type, Herb. Par.).

LIPOCHAETA ROCKII **dissecta** var. nov.—Folia marginato-petiolata, principalia 5–7.5 cm. longa, plerumque 3–5-partita lobis lineari-oblongis acriter inciso-dentatis vel pinnatifidis.

Specimens examined: *Wm. Hillebrand*, western Maui (Herb. Berl.); *idem* 27, Isl. Maui (Herb. Gray; Herb. Kew; *cum L. heterophylla* A. Gr. *commixta*); *U.S. Exploring Expedition under Capt. Wilkes*, eastern Maui (type, Herb. U.S. Nat.: cotype, Herb. Gray; *cum L. heterophylla* A. Gr. *commixta); eadem, sine loco sed sine dubio* western Maui (Herb. N.Y. Bot. Gard.); *eadem*, western Maui (Herb. U.S. Nat.; *cum L. heterophylla* A. Gr. *commixta*).

The specimens from western Maui have the leaf lobes less finely dissected but are hardly to be separated varietally.

L. rockii and its varieties may be separated according to the following key:

a. Folia plerumque 3–5-partita
  b. Foliorum lobi lineari-oblongi acriter inciso-dentati vel pinnatifidi, planta mauiensi . . . . var. *dissecta*
  b. Foliorum lobi latiores moderate dentati rare paulo incisi, planta molokaiensi et oahuensi . . . . *L. rockii* sensu stricto
a. Folia grosse dentata vel inciso-lobulata, planta molokaiensi var. *subovata*

Lipochaeta intermedia Degener & Sherff, sp. nov.—Fruticosa, foliosa, ramosa, gracilis. Folia opposita petiolata petiolis tenuibus 8–14 mm. longis, petiolo adjecto 3–5.5 cm. longa, simplicia, minute acriterque serrata, deltoideo-lanceolata, basi late cuneata vel rotundata, apice moderate acuminata, crassiuscula, utrinque incano-hispida, subtus valde elevato-venosa. Capitula numerosa corymboso-cymosa, gracillime pedicellata pedicellis usque ad 2 cm. longis, radiata, pansa ad anthesin 1.2–1.6 cm. lata et circ. 5–6 mm. alta. Involucri hispidi bracteae exteriores circ. 4 vel 5, nunc oblongo-lanceolatae nunc ovatae, apice saepius subacutae, $\mp$4 mm. longae. Flores ligulati plerumque 5, flavi, ligula obovato-oblongi, apice subintegri, tantum circ. 6–7 mm. longi. Achaenia atra, cuneata, circ. 2.3–2.5 mm. longa, exteriora 3–4-gona, saepe tumida, glabra vel angulis obsolete muriculata, supra saepe spinosa et centraliter 1–6-aristata aristis brevibus tenuibus antrorsum hispidulis.

Specimens examined: *Alfred Meebold* (*Degener* distrib. no.) 4254, among lava, Huehue, Kona District, Isl. Hawaii, Hawaiian Isls., May, 1932 (type, Herb. Field Mus.).

A species somewhat intermediate between *L. lavarum* (Gaud.) DC. (especially the var. *ovata*) and *L. subcordata* A. Gray. From *L. lavarum* it differs at once in its smaller and much more numerous capitula; from *L. subcordata* it is easily distinguished by its more slender and basally wide-cuneate or rounded (not subcordate or cordate) leaves, etc.

Lipochaeta tenuis Degener & Sherff, sp. nov.—Herbacea vel vix suffruticosa, caulibus plus minusve procumbentibus gracillimis elongatissimis angulatis glabratis vel sparsim adpresso-hispidulis $\mp$1 m. longis. Folia tenuiter petiolata petiolis circ. 1–1.5 cm. longis petiolo adjecto circ. 3.5–5.5 cm. longa, indivisa, tenera, deltoideo-lanceolata vel parce deltoideo-ovata, subtus pallida, utrinque minutissime ad-

presso-hispidula, marginibus acriter serrata circ. 7–9 dentibus pro unico latere, apice acuto mucronata. Capitula perpauca, tenuiter pedunculata pedunculo ∓3 cm. longo, radiata, pansa ad anthesin ∓2.6 cm. lata et circ. 6 mm. alta. Involucri pallidi bracteae exteriores 4 vel 5 oblongo-ovatae vel oblongo-lanceolatae apice acutae ∓6 mm. longae. Flores ligulati ∓10, intense aurei, ligula lineari-oblongi, apice interdum denticulati, circ. 1.1–1.3 cm. longi. Achaenia primum aristata demum (unicum maturum exterius visum) apice calva, cuneato-obovata, brunnea, trigona, obscure verrucosa, angulis aegre vel obsolete interrupto-alata, apice minutissime setulosa, circ. 2.4 mm. longa.

Specimens examined: *Otto Degener*, a long, straggling, suffrutescent herb in the rain forest, Waianae Valley, up toward Mt. Kaala, Isl. Oahu, Apr. 24, 1932 (type, Herb. Field Mus., 2 sheets: cotype, Herb. Kew).

CHICAGO NORMAL COLLEGE

STRUCTURE AND DEVELOPMENT OF PILULARIA MINUTA. DURIEU MANUSCRIPT[1]

DUNCAN S. JOHNSON

(WITH FORTY-FOUR FIGURES)

Introduction

This diminutive species of the Marsileaceae, first discovered by DE NOTARIS in 1835 at Pula in Sardinia, has since that time been collected at two other stations in that island, in Algeria, in southern France, and in Smyrna (4, 7). Although thus rather widely distributed about the Mediterranean it has, perhaps as a consequence of its small size and its delicacy, only rarely been collected, its structure has been but little studied, and its development has not been described at all.

Because the capsule of *Pilularia minuta* has but two sori, the smallest number known in the whole family, the writer has long wished to compare its development and detailed structure with those of the capsules of *P. globulifera* and *Marsilea quadrifolia* (JOHNSON 10, 11). The first opportunity to do this came recently, when herbarium specimens of this plant collected by DURIEU in Algeria were received on loan from the National Herbarium, the New York Botanical Garden, and the University of Montpellier, and when fruiting plants preserved in alcohol were received from Professor J. PAVILLARD of Montpellier. Grateful acknowledgment is here made to the institutions mentioned, and above all to Professor PAVILLARD for the valuable alcoholic specimens of the plant.

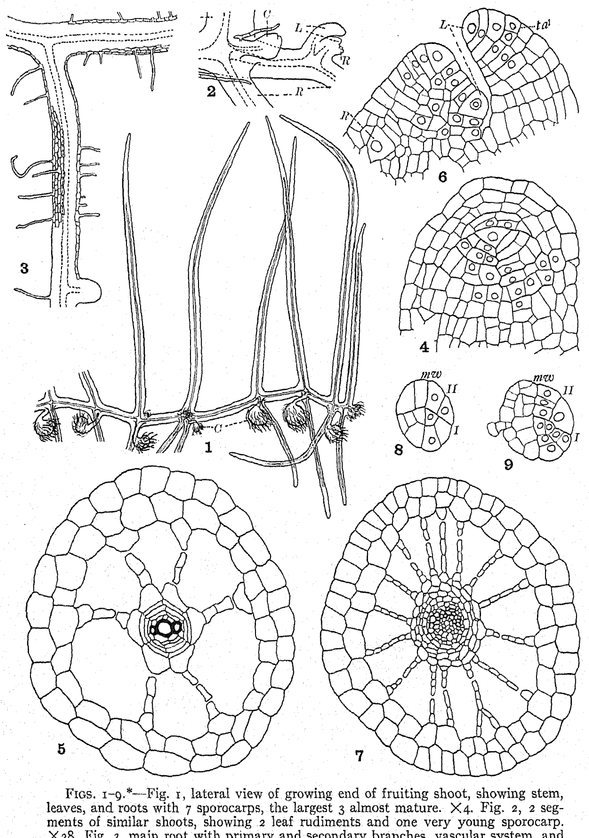
From the study of whole shoots mounted in glycerin, of hand sections of capsules, and especially of paraffin sections of all available alcoholic material of the stem, leaf, and sporocarp, many of the essential features of the later development of these organs have been determined. Unfortunately the very earliest stages of development of the sporocarp could not be found.

[1] Botanical contribution from the Johns Hopkins University, no. 117.

Vegetative structure

The stem of this most delicate of all the Marsileaceae is approximately 4–6 cm. long, 0.5–0.7 mm. in diameter, and consists of twelve or more segments. Each node of the stem bears a single leaf, one or two roots, often a bud or branch, and, when fruiting, a single sporocarp (figs. 1, 2). The individual internodes of the mature parts of the stem attained a length of from 3 to 6 or 8 mm. The internodes of the stem had an average thickness of 500–700 μ. The stele of the internode is less than one-fifth the diameter of the stem itself. The initial of the stem is probably three-sided although this was not determined with certainty (fig. 6). The younger parts of the stem are well protected by very numerous trichomes. The adventive roots of this species are oftenest simple, but in certain cases there are branches of the second and third orders. The longest simple root seen was 20 mm. in length, and the primary branch shown in figure 3 was 16 mm. long. Root hairs were abundant (fig. 3) although the roots seemed entirely free of solid soil particles. The root initials arise near the stem apex, approximately opposite (that is, below) those of the leaves. The root initial is tetrahedral (fig. 4) and gives rise to a well-marked root cap in addition to the tissues of the root proper. The average diameter of the root is about 150 μ, and that of its vascular bundle about 30 μ.

The leaf is often 35–40 mm. in length and has a diameter of 500–700 μ. Its vascular bundle is little more than 100 μ in diameter. The leaf arises from a single, evidently two-sided initial which is formed on the upper surface of the stem, apparently to the right or left of the mid-line (fig. 6). The total number of segments finally cut off by this initial could not be traced definitely, as was done in the case of *Marsilea* (10). Approximately transverse sections near the tip of the leaf show, however, that the segments of the initial are practically semicircular discs (fig. 6), just as they are known to be in *Marsilea* and *Pilularia*. Moreover, the earlier longitudinal anticlinal walls dividing these segments, the "section walls" of JOHNSON (10, 11) have precisely the position that they were shown to have in *Marsilea quadrifolia* and *Pilularia globulifera* (figs. 8, 9). Cross-sections of the mature leaf seem to show clearly that the epidermis, mesophyll, and vascular bundle are differentiated much as they are



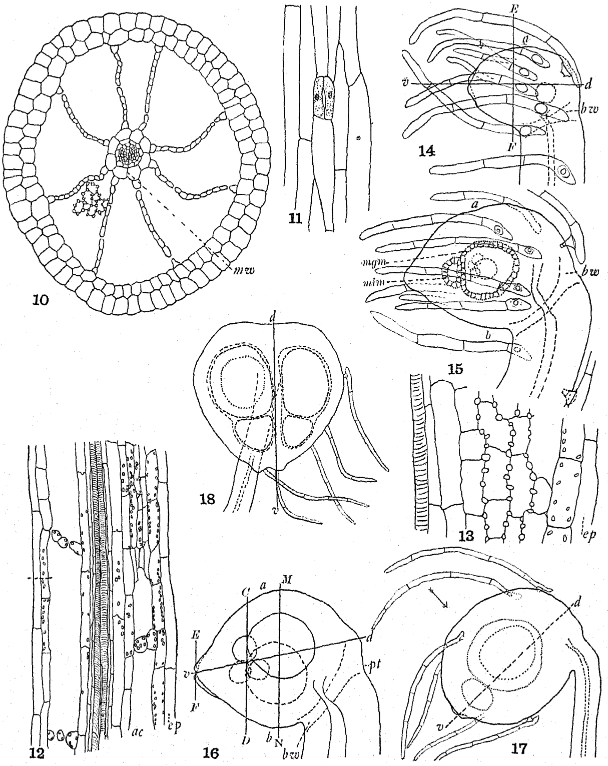
FIGS. 1–9.*—Fig. 1, lateral view of growing end of fruiting shoot, showing stem, leaves, and roots with 7 sporocarps, the largest 3 almost mature. ×4. Fig. 2, 2 segments of similar shoots, showing 2 leaf rudiments and one very young sporocarp. ×28. Fig. 3, main root with primary and secondary branches, vascular system, and root hairs. ×42. Fig. 4, longitudinal section of young root apex showing initial and root cap. ×350. Fig. 5, transverse section of mature root showing 6 air canals, 6 endodermal cells, and very simple vascular strand. ×350. Fig. 6, longitudinal section (approximately sagittal) of stem apex showing initial of stem, those of 2 young leaves, and root initial. ×350. Fig. 7, transverse section of nearly mature stem showing 15 air canals and structure of stele. ×180. Fig. 8, approximately transverse section of tip of young leaf showing semicircular form of segments of leaf initial and (at I and II on right) positions of first longitudinal anticlines dividing this segment. ×350. Fig. 9, similar section of leaf just older than that in fig. 8, showing (at right) earlier longitudinal walls in one segment of leaf initial. ×350.

* Abbreviations: *a*, apex; *abax*, abaxial side of bundle; *ac*, air canal; *adax*, adaxial side of bundle; *b*, basal side of capsule; *bw*, basal wall; *C*, capsule of sporocarp; *d*, dorsal; *ep*, epidermis; *id*, indusium; *L*, leaf; *mast*, macrostoma; *mb*, meridional bundle; *mc*, marginal cell; *mgm*, megasporangium; [illegible] microsporangium; *mw*, median wall; [illegible]

in *Marsilea*. Early in its development the leaf grows more rapidly on the dorsal side, and in consequence pushes around in front of the stem growing point. It later continues this differential growth to form the characteristic watchspring-like, circinately coiled leaf. A cross-section in the mature leaf shows that the epidermis is supported by a well developed hypodermis beneath which lie eight relatively large, longitudinal air canals separated by perforate radial partitions (figs. 10, 12). The minute vascular bundle consists of relatively few vascular elements, usually but two tracheae, and is surrounded by a weakly developed endodermis. The trichomes are deciduous, so that the mature leaf is naked except for the remnants of the second cell of the trichome which sometimes adheres to the persistent basal cell that lies imbedded between the normal epidermal cells. The stomata of the leaf are relatively few. The guard cells are elongated (with the leaf) to four or five times their transverse dimension (fig. 11).

Sporocarp

The sporocarps of *Pilularia minuta* are attached to the stem, a single sporocarp in front of the base of each leaf. The relatively elongated but slender peduncle of the mature sporocarp may be 1.5–2.5 mm. in length. In general young sporocarps are those nearer the stem apex, although some of those found farther from the apex are smaller, probably because retarded in development. The sporocarp even at maturity is densely covered with glistening, tawny hairs, so that it is not always easy to determine its exact external form (figs. 1, 2, 14). The capsule of the sporocarp is more or less ovoid, often decidedly flattened on the side toward the peduncle (figs. 23, 27, 31), and the "tip" (really ventral side) of the capsule when mature is bent downward against the base or middle portion of its own peduncle (figs. 18, 21, 22). This flattening of the capsule, together with the bending of its stalk, makes it externally a distinctly dorsiventral or bilaterally symmetrical structure, a type of symmetry which is based on its mode of development and is carried out also in every detail of internal structure, as will be described later. The tip of the capsule, even when practically mature, shows two papillae, the origin of which also will be mentioned later. At the base of the capsule (that is, at the point of juncture with the peduncle



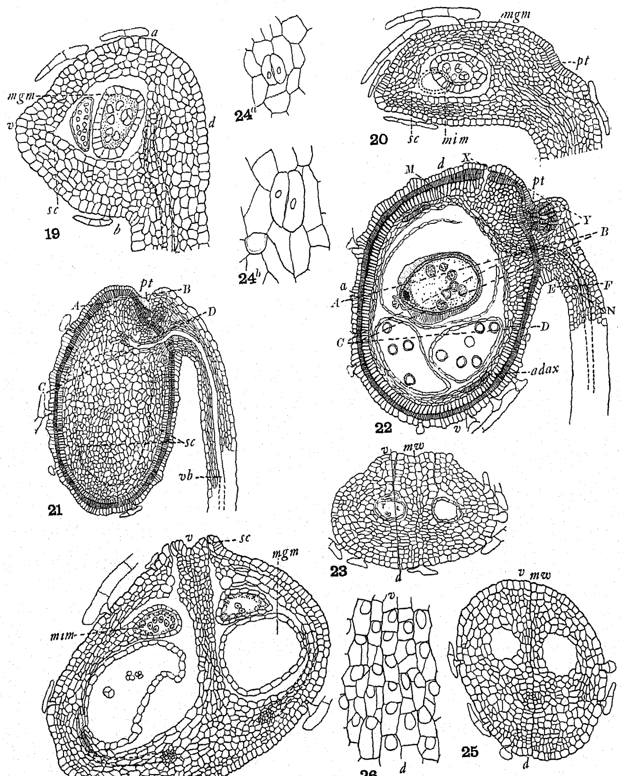
FIGS. 10–18.—Fig. 10, transverse section of mature leaf, near middle, showing vascular bundle, endodermis, 8 air canals, 8 perforated partitions, and continuous hypodermis and epidermis. ×120. Fig. 11, tangential section at surface of mature leaf showing epidermis and stoma. ×260. Fig. 12, radial section of nearly mature leaf showing vascular bundle and perforate radial and transverse partitions between air canals. ×180. Fig. 13, portion of perforate radial partition of leaf showing form of component cells. ×260. Fig. 14, left side of young sporocarp showing relatively large trichomes at this stage, form of capsule, position on peduncle, vascular bundle of latter, and basal wall between capsule and peduncle (*a*, morphological apex of capsule). ×90. Fig. 15, similar view of somewhat older capsule showing position of megasporangium and microsporangium and vascular bundle penetrating basal wall. ×90. Fig. 16, diagram of left side of capsule older than that in fig. 15, indicating sporangia of both locules and 2 papillae marking ventral ends of soral canals (*CD*, *EF*, *MN*, *vd*, planes of sections shown in several following figures). ×90. Fig. 17, left side of almost mature capsule showing double beak, dorsal pit, and tubercle of capsule. ×30. Fig. 18, approximately apical view of same capsule (arrow in fig. 17 indicates direction) showing tip of capsule and position of megasporangium and of one of the two microsporangia

and on the abaxial side) there is a distinct depression in its surface, the so-called basal (dorsal) pit; while just basal to this at the upper end of the peduncle is a marked elevation or tubercle, probably the equivalent of the tooth on the sporocarp of *P. globulifera*.

The internal structure of the sporocarp of *Pilularia minuta* is the simplest found in any of the Marsileaceae. Whether it is a primitively simple structure or not is a question that can better be taken up later, after its development has been discussed. It may be stated here that the general plan of structure of this capsule is the same as that found in *P. globulifera*, and therefore it also is regarded as a modification of the type found in *Marsilea*.

The capsule of *P. minuta*, as is clear from examination of transparent older sporocarps, of sections (figs. 18, 27, 28, 30), or of a burst capsule, consists of two locules with one sorus each. These locules lie one on each side of the sagittal plane (that is, of the median wall) that results from the repeated segmentation of the evidently two-sided initial of the sporocarp (figs. 23, 25, 27, 29, 35). Each locule has a somewhat ovoid hemispherical shell, the outer convex side of which is formed by one-half of the 6- to 8-layered outer wall of the capsule. The flat, diametric partition wall of the locule, the indusium, is composed of three to five layers of cells that arise in each half of the capsule next to the median plane. Hence the soral cavity in each locule is separated from its fellow of the other half of the capsule by the two plane walls, each three to five cells in thickness (figs. 21, 23, 28).

Each locule (or half) of the capsule bears a single placenta, which forms a small mound of thin-walled cells projecting into the soral cavity from about the middle of the outer wall of each half of the capsule (figs. 28, 29). Each placenta is supplied by the middle one of three meridional vascular bundles that are formed in each half of the capsule. From each placenta are developed a single megasporangium, which lies near the dorsal side (or "base") of the capsule (figs. 15, 16, 18, 22, 28) and two microsporangia, which lie nearer the tip, or ventral side, of the capsule (figs. 15, 18, 22, 28). The megasporangium is about 500 μ in diameter and has a wall which at maturity is but one cell thick (figs. 19, 27, 29). The walls of the tapetal cells have by now broken down, and the contents persist, if



FIGS. 19–27.—Fig. 19, nearly sagittal section of young sporocarp (about in plane *vd*, fig. 23) showing microsporangium and megasporangium of one sorus and course (dotted) of vascular bundle from peduncle. ×156. Fig. 20, approximately sagittal (but not median) section of slightly older sporocarp showing beginning of dorsal pit and

at all, as a plasmodium-like layer about the single functional megaspore and the numerous degenerating megaspore tetrads. The microsporangia are nearly half the diameter of the megasporangium and likewise have a single layered wall (figs. 27, 28). Both microsporangia of a sorus lie at the same level and both are directed somewhat toward the tip or ventral beak of the capsule (figs. 18, 27, 28).

The single megaspore of each locule when mature nearly fills the sporangium. The many tetrads of degenerate megaspores lie about the functional one (figs. 15, 27). The megaspores are elongated, with a distinct beak but no constriction (figs. 22, 30). They average 506 μ in length and 430 μ in diameter. The wall of the megaspore is 3-layered and the wall, especially the outer layer, swells in water or potash until the diameter of the whole may reach 900 μ. The megaspore when mature contains but a single nucleus, which is large and often flattened until its diameter transverse to the spore is several times its longitudinal one (fig. 22). The cytoplasm of the whole basal portion of the spore cavity is almost completely filled with large starch grains, whose diameter is almost equal to that of the microspores of this species.

Each microsporangium contains about 128 globular microspores, each of which is about 55 μ in diameter (figs. 30, 31). The wall of

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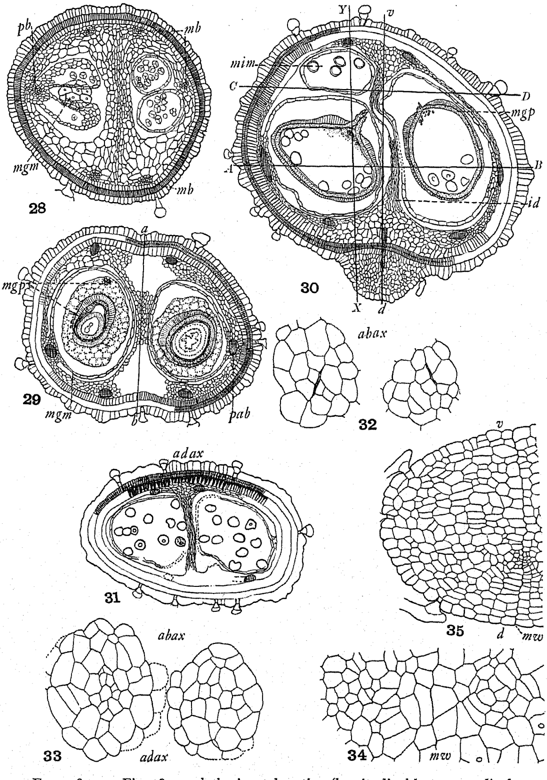
tubercle below this. $\times$120. Fig. 21, sagittal and median section (plane *dv*, figs. 18, 29, 30) of half-grown capsule showing outline of capsule, dorsal pit, tubercle, basal wall, bundle from peduncle, and the 3 specialized layers of wall of capsule. Note overlap in layers of basal wall just within pit; this median section passes through whole width of indusium between the 2 locules (cf. figs. 28, 39, 42). $\times$60. Fig. 22, approximately sagittal section of mature capsule (plane *YX*, fig. 30) showing tubercle, pit, overlapping in basal wall, course of bundle penetrating basal wall, and oblique sections of soral cavity and of 2 meridional branches of vascular bundle. $\times$60. Fig. 23, transverse section (plane *vd*, figs. 14, 16) of capsule about age of that shown in fig. 19 showing soral cavities still small, 2 soral canals, opening right and left of median wall (formed by segmentation of 2-faced initial of bilaterally symmetrical capsule (*cf. mw*, fig. 8 of leaf). $\times$180. Fig. 24, microstoma and megastoma of wall of capsule. $\times$350. Fig. 25, section (plane *CD*, fig. 21) through basal part of half-grown capsule; median wall very distinct. $\times$180. Fig. 26, part of tangential section of capsule in last figure showing epidermal cells and apical position in each of these of the rounded trichome-bearing cell formed by it. $\times$350. Fig. 27, nearly transverse section (longitudinal to locules and in plane *vd*, fig. 17) of half-matured capsule showing ventral papillae with soral canals, both megasporangia of capsule, and one of the two microsporangia of each locule. Dotted lines show course of short, middle bundle in each locule. $\times$120.

the microspore is of three distinct layers, the outer and thickest swelling greatly when soaked in water (or potash). The ripe microspore contains a single nucleus, and but few and small starch grains (figs. 22, 31).

The single vascular bundle of the peduncle is 33 μ in diameter, and consists of 35–40 cells in cross-section within the endodermis. There is no sclerenchyma strand accompanying the bundle here as in *P. globulifera* (**16**, figs. 11, 12; **11**, figs. 32, 33). This single bundle continues to be the same diameter up the middle of the peduncle to the proximal (dorsal) end of the capsule. There it bends sharply, constricts to half its former diameter, and penetrates at a right angle the thickened hypodermis or light-line layer of the basal wall of the capsule (figs. 15, 16, 21, 22). Soon after entering the capsule (figs. 22, 30) the bundle expands to its former diameter and then forks T-like to two arms which extend transversely, one into the right half of the capsule and the other into the left. Each arm after reaching a length of 60–70 μ divides to form an abaxial branch and an adaxial one (fig. 37). The former remains undivided and runs near the midplane and just within the wall of the capsule, ending near the tip of the soral canal of its locule. It thus forms one of the three meridional bundles found in each half of the capsule (figs. 22, 28, 29, 30). The adaxial branch on the contrary soon divides into two. One of these forms an adaxial meridional bundle which runs, near the median plane of the capsule on the side next the peduncle, around to the tip or beak of the capsule (figs. 22, 28, 30, 31 *adax*). The other division of the adaxial branch takes a meridional course near the middle of the locule. When it is half way to the tip, however, it turns inward to enter the placenta (figs. 27, 28, 29), and no portion of this bundle is left to run to the tip of the capsule as in *P. globulifera* (fig. 31). This feature sharply distinguishes the vascular bundle system of this species from that of *P. globulifera* and of many species of *Marsilea*, in which bundles of the same locule of the capsule fuse near the ventral side of the capsule (**4**, figs. 1–7; **10**, fig. 44; **16**, figs. 28, 29; **11**, figs. 33, 38). The vascular bundle system of this species is then decidedly simpler than that of *P. globulifera* and far simpler than that of any species of *Marsilea*.

The wall of the capsule in *Pilularia minuta* consists, as it does in



FIGS. 28–35.—Fig. 28, nearly horizontal section (longitudinal but perpendicular to median plane) (at level *CD*, fig. 16) of slightly older capsule showing rounded outline, thick median indusium, 2 microsporangia in each sorus, and tip of one megasporangium (note 3 meridional bundles of each locule, middle one ending in placenta, as shown

P. globulifera, of three outer layers of markedly specialized cells within which are three to six layers, or in some parts still more, of parenchymatous cells, among which are imbedded the vascular bundles. Certain of these parenchyma cells, chiefly those situated near the median plane (figs. 28, 29), finally swell to help burst the capsule and expel the spores. In two other regions (one at the middle of each valve of the capsule) it is the parenchyma layer of the wall of the capsule that becomes thickened to form the placenta (figs. 28, 29 *pab*).

Of the three more specialized layers of the wall proper of the capsule, the outer, the epidermis, consists of ordinary epidermal cells, of trichome-forming cells, and of the guard cells of the relatively few microstomata and megastomata found chiefly near the base of the capsule (figs. 24, 38). The epidermal cells are more or less polyhedral in outline with straight or wavy sides (fig. 26). Those forming the somewhat netlike elevations of the surface (18) are often 35 μ thick (perpendicular to the surface of the capsule), while the cells filling the meshes may be but 10–12 μ thick (figs. 22, 30). The epidermal cells below the bend of the peduncle are also strikingly elongated (figs. 21, 22).

The trichome-forming cells are numerous and are distributed with great regularity. Thus on the apical surface of the young capsule there is a circular trichome-forming cell cut out from the same (ventral) end of practically every surface cell (fig. 26). Later the remainder of the surface cell is cut by longitudinal and transverse

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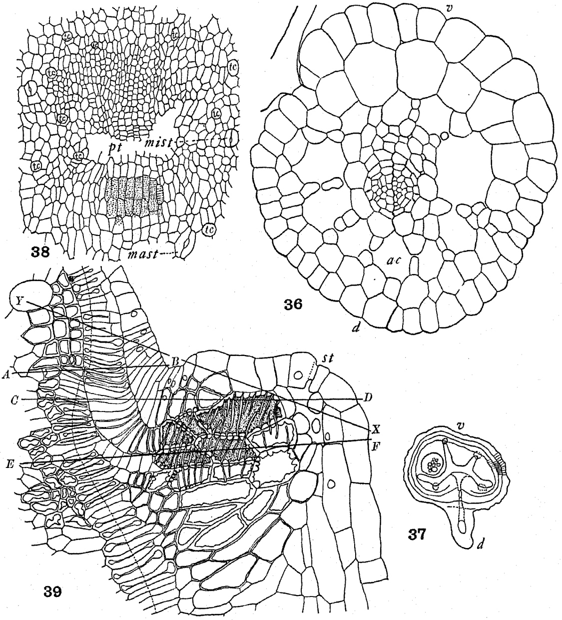
at left). ×60. Fig. 29, horizontal section of same sporocarp taken nearer the base (line *MN*, fig. 16 or *AB*, fig. 30) and thus passing through the single megaspore of each locule. Note meridional bundles, placenta, stalk, and walls of megasporangia, and (at left) plasmodium-like tapetal residue surrounding 3 degenerating megaspores and one functional megaspore. ×60. Fig. 30, obliquely horizontal section (along line *Y-C*, fig. 22) of mature capsule showing 2 ripe megaspores, each inclosed by its sporangium wall, and vascular bundle penetrating basal wall. ×60. Fig. 31, approximately horizontal section of capsule near tip (plane *CD*, figs. 22 and 30) showing microsporangia only and absence, beyond placenta, of middle meridional bundle of each locule. ×60. Figs. 32–34, 3 successive horizontal sections (transverse to soral canals) through papillae of ventral tip of half-grown capsule. Soral canals closed, indication of a canal at the center of each papilla in fig. 32; median wall marking sagittal plane shown in fig. 34 at *mw*. ×350. Fig. 35, one-half of transverse section near middle of young peduncle with vascular bundle just differentiating; median wall clear. ×350.



FIGS. 36–39.—Fig. 36, transverse section of older peduncle, immediately below capsule, showing median wall, 8 air canals, and vascular bundle with well marked endodermis. ×350. Fig. 37, obliquely horizontal section (plane *MN*, fig. 22) through base of capsule showing bilateral forking of vascular bundle after entering capsule and subsequent divisions of each fork to form (as on left) 3 meridional bundles in each locule. ×30. Fig. 38, tangential section (on line *XY*, figs. 22 and 39) of pit at base of capsule showing outline of pit, character of cells of tubercle and of basal wall as cut in this plane; also distribution of trichomes and of guard cells of 3 microstomata and of one megastoma. ×180. Fig. 39, basal (dorsal) portion of median sagittal section of mature capsule showing structure of tubercle, lining of basal pit, and overlap of hypodermis in basal wall. ×350.

anticlines (fig. 26) to form four or more of the epidermal cells of the mature capsule (*cf.* **10**, figs. 2, 3, 15, 16).

The mature trichome consists of some six or seven cells altogether. The innermost is the basal cell which is about half the height of the surrounding epidermal cells and has a rather thick wall (figs. 15, 17, 22, 30). Outside of this is the funnel-shaped stalk cell on the outer end of which is borne the basal cell of the 4- or 5-celled, ventrally directed, tapering limb of the trichome (figs. 14, 17, 20). The slightly thickened but firm cell walls of the limb are pale brown and give this color (somewhat lightened by the overlapping of these nearly transparent hairs) to the ripe, dry capsule itself. The form and character of the walls of the components of the two distinct layers of palisade-like cells next below the epidermis have been but briefly described from this species by RUSSOW (**17, 18**). A fuller discussion of these layers in *P. globulifera* is given by METTENIUS (**15**), RUSSOW, and especially by MEUNIER (**16**). The outer of these two prismatic layers, the hypodermis or light-line layer, is in *P. minuta* faintly yellowish in natural color, and stains a pale blue with haematoxylin. Over most of the capsule this layer is 15–20 μ thick; that is, the component cells are 15–20 μ long (figs. 22, 30). In a considerable region above the pit, at the upper end of the peduncle, however, these cells may become 35–40 μ long (figs. 22, 39). Certain peculiarities of this layer in the basal part of the capsule will be mentioned later. The inner of the two prismatic layers of the wall, the subhypodermal layer, is composed of cells of much less regular form and size. Their radial length ranges from 30 to 40 μ and their diameter is half this or less. On the flanks of the capsule these cells are rather regularly prismatic, but toward the base of the capsule, especially at and above the point of penetration of the vascular bundle into the capsule, the single layer of these brown prismatic cells is replaced by several irregular layers of similarly thickened brown cells which here become packed together promiscuously (figs. 22, 30, 39).

There are several strikingly specialized layers or groups of tissues in the dorsal (basal) portion of the wall of the capsule, near the median plane and just within the tubercle. These structures are important not only because they furnish further evidence of the complete zygomorphic symmetry of this capsule, but also because they

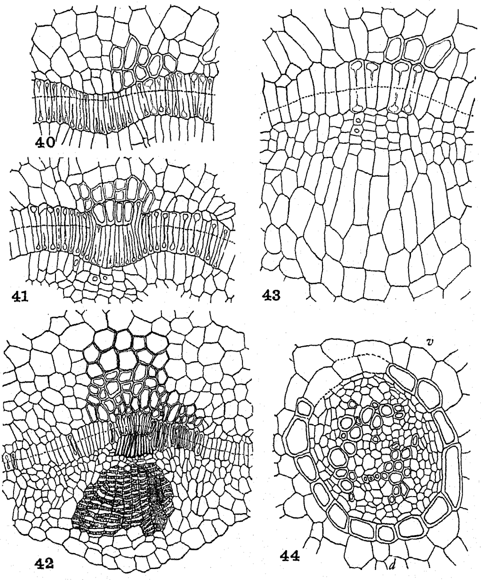
show its similarity, even in these minor details, to that of *Marsilea.* These specialized structures are as follows:

1. There is a close overlapping of a distinct median flap of the outer prismatic layer (hypodermis) as it comes down from the upper side of the capsule, upon a narrow, attenuated tongue, the same prismatic layer which here forms a firm wall across the dorsal end of the capsule (figs. 21, 22, 39, 41, 42). As will be seen from these figures, this flap is only eight or ten cells wide, and the more refractive zone which usually forms the light-line across the middle of each hypodermal cell has here, at the overlap, moved toward the inner ends of these cells, finally to disappear before quite reaching their inner ends (figs. 39, 40, 41). These cells of the outer overlapping layer are continued, beyond where the light-line disappears, by a layer of prismatic cells which are still more elongated but are thin-walled and distinctly curved. This layer finally bends outward to abut upon the hypodermal or subhypodermal layer of thickened cells of the tubercle (figs. 21, 39). The tongue of hypodermal cells that runs up from below to underlap the flap from above may be only five or six cells wide (fig. 41), and a similar shift of the light-line occurs here. In this case, however, it is toward the outer ends of the cells (figs. 22, 39, 42). The cells of this tongue also, beyond the middle of the overlap, are thinner-walled; but instead of becoming longer radially they divide to form two layers of cells, each a half or a third the usual thickness, and finally the inner of these layers abuts upon a layer of thick-walled, brownish, subhypodermal cells (fig. 39).

2. Another peculiar tissue found at the base of the capsule is a considerable group of thin-walled, radially elongated, epidermal cells that forms the bottom of the pit, just above the tooth (figs. 19, 38). These differ markedly from the shorter, brown-walled cells composing the rest of the epidermis. They gradually decrease in length and, above, they finally abut upon a single layer of epidermal cells, while below they are replaced by two layers of similarly thin-walled, colorless cells just above the tubercle (fig. 39).

3. In the basal "tooth" or tubercle of the capsule occurs a third type of specialized cell, namely, the radially elongated scalariform, thickened cell. This forms a group about 8–10 cells in width and 6–8 cells in vertical depth (figs. 22, 39, 42). The character of the

thickenings on the walls of these cells suggests that they may be water-storage tracheids or perhaps have mucilaginous walls which at germination swell to enlarge this part of the capsule and thus



FIGS. 40–44.—Fig. 40, portion of obliquely horizontal section (perpendicular to basal wall) just above line *AB* in fig. 39 showing outer overlapping layer of thickened cells as continuous around whole base of capsule. ×180. Fig. 41, portion of similar section but at level *AB* of fig. 39 and so showing overlap of two layers of hypodermis in basal wall. ×350. Fig. 42, portion of similar section, but near level *CD* in fig. 39, showing tubercle and inner (lower) wall continuous at overlap. ×180. Fig. 43, portion of section like last but at level *EF* in fig. 39, showing continuous single basal wall below overlap. ×350. Fig. 44, transverse section of stele of mature stem showing endodermis, vascular elements, and radial partitions of cortex. ×350.

permit water to enter the capsule itself and so to aid in opening the latter.

4. The characteristic aerating tissues of the capsule are far more abundant in the outer part of the tubercle itself and just ventral to the basal pit than elsewhere. Stomata are present about (and especially above) the pit, and they open into deep air canals that penetrate through both prismatic layers to the relatively abundant intercellular spaces between the underlying parenchyma cells (fig. 22). There are some stomata also on the dorsal side of the peduncle which open into the longitudinal air canals in this stalk and thus connect with the intercellular spaces of the tubercle itself (fig. 22).

All of the four groups of tissues just described are located on or near the dorsal line of the capsule or peduncle. In other words, they all lie in or near the plane of symmetry and so all serve further to emphasize the zygomorphic character of the sporocarp, which is, as previously seen, already clear from the arrangement of the vascular bundles, the soral canals, the indusia, and the sporangia.

Concerning the functions of the structures found at the base of the capsule, it is seen from what has been said that both layers of hypodermal cells, at the overlap of these in the base of the capsule, are decidedly thinner-walled than they are elsewhere. Since the neighboring cells of the epidermis (fig. 39) are likewise unthickened, it is clear that this is the one area of the whole wall of the capsule most readily permeable to liquids. The presence of air spaces among the cells of the tubercle, and of others connected with the stomata above the dorsal pit, indicate that this portion of the wall of the capsule is also the one through which gas interchange with the atmosphere can most readily occur. It also seems possible, from the readiness with which the cells of the two overlapping layers separate along the plane of contact, that gases may, under certain conditions of distention or shrinkage of the surrounding tissues, actually pass through this intercellular slit to or from the capsule proper.

Development of capsule

Unfortunately it has not proved possible to find the single initial of a sporocarp at the base of the leaf such as was demonstrated in the case of *Marsilea quadrifolia* (10, figs. 22, 23). Nor was it possible, as

in the other Marsileaceae studied (**10**, **11**), to secure longitudinal and horizontal sections of a sporocarp of *P. minuta* young enough to show its continuing growth by a single bifacial apical cell (*cf.* **10**, figs. 34, 38 and **11**, figs. 13, 14). But the mode of growth of the leaf of *P. minuta* is clearly identical with that demonstrated in *Marsilea* and *P. globulifera* (*cf.* figs. 5, 6 with **10**, figs. 2, 6, 7 and **11**, figs. 3, 4, 5). Moreover the cell arrangement in cross-sections of the youngest obtainable capsules of *P. minuta* (figs. 23, 25, 35) corresponds closely with similar sections of young capsules of the other two Marsileaceae mentioned (**10**, figs. 32, 33 and **11**, figs. 19, 24, 26). It therefore becomes practically certain that the earlier steps in the development of the sporocarp of *P. minuta* must correspond essentially with these same stages of *P. globulifera* and so with those in *Marsilea quadrifolia*.

Thus cross-sections of the young capsules of *P. minuta* show a diametric median wall, identical in position, and probably in origin, with the median wall found in capsules of *P. globulifera* (figs. 23, 25). This median wall, which in *P. globulifera* was shown to mark the line of juncture of the successive segments cut from the right and left faces of the bifacial initial, is seen clearly not only in cross-sections of the fertile portion or capsule of the sporocarp of *P. minuta*, but it also stands out distinctly in cross-sections of the peduncle (figs. 35, 36), and even in horizontal sections of the sterile tip, or of the base of the capsule (figs. 25, 34). This median wall evidently results from segmentation of a 2-sided initial, which is obviously the developmental foundation for the bilateral or zygomorphic symmetry of this sporocarp. The median wall lies in the plane of symmetry (the sagittal plane) which passes vertically between the two sori in a face view of the capsule (fig. 18); in a horizontal or cross-section it passes vertically through the indusium that separates the two soral cavities (figs. 23, 25, 27, 29). A median sagittal section of the capsule will not touch either soral cavity, therefore, but will cut through the indusium for its whole length (figs. 21, 28).

Further evidence of the bilateral symmetry of this capsule is seen in the pair of soral canals and the two ventral papillae usually marking the openings of these canals in half-grown capsules (figs. 14, 16, 17, 18, 27, 33). Comparison of such figures with figures of the same

stages in *P. globulifera* (**11**, figs. 19, 24, 25, 26) leaves no doubt that the six sporangia of the two sori of *P. minuta* have arisen, as was demonstrated for the more numerous sporangia of the four sori of *P. globulifera*, from originally superficial marginal cells of the ventral side that have been secondarily buried by the more vigorous growth, ventralward, of the surrounding cells, the cells that are to form the indusium and the wall of the capsule.

The development and gradual specialization of the cells composing the vascular bundles and the various parts of the wall of the capsule, including the tubercle and pit, are indicated in the figures of capsules of different ages (figs. 19, 20, 21, 23, 28, 39). The development of the wall and bundles has been described for *P. globulifera* by MEUNIER (**16**), who failed, however, to note the basal tubercle or pit. One developmental feature that should be emphasized here is the curvature of the terminal portion of the peduncle. This is important because it determines the position of the mature capsule on the peduncle, which is different from that found in *P. globulifera* and from those found in most other Marsileaceae. In the two youngest sporocarps seen (figs. 14, 19), both of which already had well developed sporangia, the capsule portion stands nearly at right angles to the peduncle. It is believed that a very young sporocarp would, like that of *P. globulifera* (**11**, figs. 14, 31), show the peduncle and capsule lying in one straight line with the soral canals facing ventrally at right angles to this line. As development progresses, the dorsal side of the peduncle, near its upper end, continues to grow more rapidly than the ventral side (figs. 14, 16, 17), with the final result that the capsule is bent through 90° (figs. 17, 18) until it lies almost flat against the ventral side of its peduncle. Its closed soral canals thus come to point toward the stem at the base of the peduncle, instead of at right angles to this direction as they did earlier (figs. 18, 20, 22). This is the reverse of the position on its peduncle of the mature capsule in *P. globulifera*, in which the tips of the canals finally point very nearly upward (**11**, figs. 32, 33, 36). Another result of this curvature of the peduncle is that the morphological apex of the capsule, the location occupied by the apical cell when it was active, comes to lie on the side of the capsule away from its own peduncle (fig. 22; *cf.* **11**, figs. 14, 31, 33*A*). The different degrees of such curva-

ture of the peduncle found in other species of the Marsileaceae, with some of the misconceptions to which these have led, will be discussed in a later paper.

Although this bending is in the same direction as the circinate bending of the tip of the leaf, it is not really the equivalent of this, since the bending is confined to a single fixed zone of the peduncle, not to a constantly advancing zone. The terminal portion of this structure (the capsule itself), moreover, does not bend at all.

Development of the sporangia in *Pilularia minuta* could not be followed through from the beginning since the youngest sporocarps had sporangia with well formed, single-layered walls and with either spore mother cells or tetrads (figs. 15, 19, 20) already differentiated. These and the later stages seen offered no evidence of essential differences in the development of the sporangium and spore from those already described in detail (**16**, **6**). All the sporangia initiated mature and produce functional spores. There is no indication of even the initiation of more than one megasporangium and two microsporangia per sorus, which might suggest the derivation of this sporocarp from one with a larger number of sporangia like that of *P. globulifera* or of *Marsilea.*

The structure and development of the capsule of *P. minuta*, as here described, indicate its close correspondence with that of the capsule of *P. globulifera*. Like the latter it is similar in all essential respects to the capsule of *Marsilea quadrifolia*. The adherence of this capsule of *P. minuta* to the *Marsilea* plan is shown by the equivalent dorsiventral (zygomorphic) symmetry; by the arrangement of the placentas and their vascular bundles; by the structure of the dorsal pit and tubercle; and by the overlapping of two layers of the thickened hypodermis inward from the pit. Finally the study of all the stages of development that were available showed these to correspond essentially to the homologous stages of the capsule of *P. globulifera* and so to those of *Marsilea*. This comparison of the capsule of *P. minuta* with those of other Marsileaceae shows it to be the simplest one of the family. This simplicity is indicated: by the reduction of the number of sori to two, the smallest number possible if the zygomorphic symmetry of the *Marsilea* type of sporocarp is to persist at all; by the reduction of the number of megasporangia in

each sorus to one and of microsporangia usually to but two; and by the correlated reduction of the number of branches of the vascular bundle in the capsule and the lack of anastomoses of the ventral tips of the branches such as occur regularly in *Marsilea* and in other species of *Pilularia*. It is thought that this simplicity is a secondary one that has been derived by a process of reduction from a type of vascular system like that of *Marsilea quadrifolia* or *M. brownii*, and that forms like *M. aegyptiaca* (3), *Regnellidium* (14), and *Pilularia globulifera* illustrate successive stages in this reduction process. There seems adequate reason also, from the structure of the leaf as well as of the sporocarp, for believing that *Marsilea* species with four pinnae and numerous sori, like *M. quadrifolia*, are the most primitive members of the family Marsileaceae (2).

Summary

1. Although *Pilularia minuta* has been known for a century it is rarely collected and has been little studied, its development practically not at all.

2. The plant is small and has a delicate stem and a leaf but half a millimeter thick and a few centimeters long. Each node of the stem bears a leaf, a bud or branch, one or more roots, and, when fruiting, nearly every one also bears a single sporocarp. The vascular bundle of the latter is attached directly to that of the stem, close beside that of the leaf. The initial cell of the root and probably that of the stem also is tetrahedral, while the initial of the leaf is bifacial. The leaf shows no rudiment of a lamina at any stage of its development. Delicate trichomes, each with a 2-celled stalk and a tapering limb of 4–6 square-ended cells, cover all young organs except roots.

3. The sporocarp is rather long stalked, with a brownish, ovoid capsule, the tip of which, by a bend of the peduncle, is finally brought to face toward the base of the latter. The capsule has a basal pit and a tubercle just below it, at the very end of the peduncle; while on the opposite side of the capsule, at the ends of the soral canals, are two nipple-like papillae. There is but one sorus on each side (in each locule) of the bent capsule, each with one megasporangium toward the base and two microsporangia toward the tip. The single megaspore of each sorus is uninucleate when mature, and

is densely stored with large starch grains. The fibrovascular system of each of the two locules consists of three meridional branches that do not fuse at their ventral ends as in most other Marsileaceae. In fact the middle branch in each locule ends altogether at the placenta, half way to the tip of the capsule.

4. The firm wall of the capsule consists of a slightly thickened epidermis and the hypodermal and subhypodermal layers of much thickened prismatic cells. The hypodermis is doubled, by the overlapping of two narrow strips on the median line, just above the place where the single vascular bundle from the peduncle penetrates the base of the capsule. Both peduncle and capsule of the young sporocarp show that they have been developed by the activity of a bifacial initial, like that actually observed in the leaf of this species.

5. The indusium and the lateral walls of the capsule, which together surround the soral cavity and thus finally bury the sporangia deep in the capsule, are evidently formed, as in *Pilularia globulifera*, by the more rapid growth ventralward of all cells of the ventral side of the capsule except the sporogenous cells themselves.

6. The zygomorphic symmetry of the sporocarp is unmistakable. It is evident in such external features as the bending of the peduncle; the flattening of the capsule against its peduncle; the median position on its surface of the tubercle and the pit at its base; and the pair of soral papillae at the tip of the capsule. This symmetry is also emphasized by the whole internal structure of the capsule, for example: by the right and left sori with the indusium about each; by the forking of the single median vascular bundle just after entering the capsule; by the median position of the overlap of the hypodermis in the basal wall of the capsule; and finally this same bilateral symmetry is evident through the whole history of development as far back as this could be traced. It is because the likewise unmistakable bilateral symmetry of the capsule of *P. globulifera* has been almost universally overlooked that the symmetry of the present species has been so emphasized.

7. The plan and structure of the capsule of *Pilularia minuta*, when compared with that of *P. globulifera*, indicate close correspondence in nearly every point except those in which such correspondence is rendered impossible by the differences in the size of the capsules, in

the number of sori, and in the number of sporangia in each sorus. Thus the basal pit and the tubercle below this; the peculiarities of the epidermal, hypodermal, and subhypodermal layers; and finally the overlapping of the hypodermis at the base of the capsule of *P. minuta*, all closely resemble in character these same structures as they are developed (although generally overlooked) in *P. globulifera*. The branching of the vascular bundle in each locule of the capsule of *P. minuta* is on the general plan of that in *P. globulifera* but decidedly simpler. In many of these features also this capsule resembles that of *Marsilea quadrifolia*, although the similarity here is not so close as that between the two species of *Pilularia*.

8. In general conclusion, it is evident that *Pilularia minuta* is the simplest of all the Marsileaceae; and comparison with other species indicates that it is the most reduced member of this family. Its simplicity is indicated, in the first place, by the vegetative organs, including their size and external and internal differentiation. In the second place it is shown by such features of the structure of the sporocarp as (1) the presence of but two sori, the smallest number possible if the zygomorphic symmetry of the *Marsilea* type of sporocarp is to persist at all; (2) the reduction of the number of megasporangia in each sorus to one and of the number of microsporangia to two; and (3) the correlated reduction in the number of branches of the vascular bundle in the capsule and the lack of anastomoses of the ventral tips of certain of these branches (such as occur regularly in *Marsilea* and in all other known species of *Pilularia*). With all this simplification, its adherence to the general plan as in *Marsilea* is still shown by its clearly dorsiventral (or bilateral) symmetry; by the detailed structure of the basal pit and tooth; and by the overlapping of two layers of the thickened hypodermis beneath the basal pit. Finally, study of all the available stages of development showed these to correspond essentially to equivalent stages of the capsule of *P. globulifera*, and so, of course, to those of *Marsilea*.

9. The writer believes that this simplicity in structure of the sporocarp of *P. minuta* is a derived one, which has probably been reached by a reduction from the more primitive, although more complex, type found in the capsule of *Marsilea quadrifolia* and *M. brownii*. This reduction is evident in the small size of the capsule

and in the general plan and the internal differentiation of the capsule as a whole. Perhaps, however, it is most clearly indicated by a comparison of the vascular bundle systems of the capsules in different species of this family. The simplest bundle system in the whole family is that just described for *P. minuta*, in which each locule has two complete but not anastomosing meridional branches and one incomplete branch that ends in the placenta of the sorus. This is regarded as derived ultimately from the much more complex and more primitive one of the species of *Marsilea* referred to. The different types of bundle system found in the capsules of such species as *M. polycarpa* (4), *Regnellidium* (14), *M. aegyptiaca* (4), and *P. globulifera* (16) probably illustrate successive steps in this process of simplification or reduction of the bundle system, which ends, so far as is now known, with the bundle system of *P. minuta.*

10. In confirmation of the implications of this evidence from the fertile segment of the leaf, it may be recalled that the external differentiation of the foliage leaf in the Marsileaceae has been generally admitted to indicate definitely that *Marsilea*, whose petiole or rachis has four alternating pinnae at the end, is the most primitive (that is, most fernlike) genus of the family (2). *Regnellidium* with but two pinnae, and *Pilularia* with no pinnae at all, must then be regarded as exemplifying downward steps, or reductions, in the evolution of the leaf in this family. It is clear, therefore, that the common trend of these several different sorts of evidence points to *P. minuta* as the most reduced, on the whole, of all the known Marsileaceae.

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FUSARIUM WILT AND CORM ROT OF FREESIAS[1]

J. J. TAUBENHAUS AND WALTER N. EZEKIEL

(WITH TWENTY-FIVE FIGURES)

Introduction

The growing of freesias for cut flowers has developed into a highly specialized enterprise. The parent corms are planted in the open during the late winter or early spring months in many southern states. The new crop of corms, harvested in early summer, is then shipped back to eastern and western states and planted in greenhouses to produce flowers largely for the Christmas trade. According to MORGAN,[2] florists alone purchase from 20 to 25 million corms each year for forcing purposes. Further large numbers are sold for home and estate plantings. Frequently as many as 50 million freesia corms are sold in the United States during the year.

During 1924, some Indiana growers attempted for the first time to produce their supply of freesias from Texas-grown corms. Plantings were made for several years at Donna (Hidalgo County) in the Lower Rio Grande Valley. After encouraging results at first, the crops were attacked in 1926, and even more seriously in 1927, by a disease which caused heavy losses. In the study of this disease, the writers have been assisted by growers at Donna, Texas, who have furnished land and labor, and by florists in Indiana, California, and Florida, who have furnished corms for inoculation purposes.

There appears to be little or no previous information about this disease in the literature. HEALD[3] reported a bulb rot of common freesias which he considered due to a species of *Fusarium*. Diseased corms from Texas have been submitted to Dr. HEALD, and in

[1] Published with the approval of the Director as Contribution no. 239, Technical Series, of the Texas Agricultural Experiment Station.

[2] MORGAN, W. P., A note on the hybridization of freesias. Proc. Indiana Acad. Sci. 1930.

[3] HEALD, F. D., Bulb rot of common freesia. U.S. Dept. Agric. Bur. Plant Ind. Plant Disease Reporter (mimeographed). Suppl. 65. 421. 1928.

a letter of June 5, 1929, he stated that the freesia disease from Texas appeared different from that found in Washington.

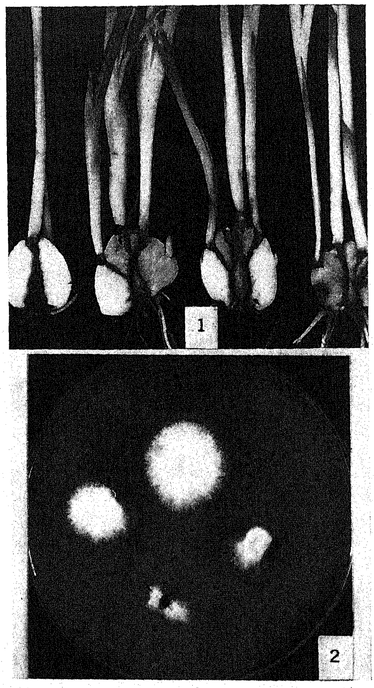
Information about freesia diseases in California was kindly furnished by Professor ELIZABETH SMITH[4] of the California Experiment Station: "Professor RALPH SMITH and I did quite a bit of work on freesias some years ago while the industry was active at Santa Cruz. . . . . The plants seemed to go down in a general rotting, usually starting at the crown and working up and down. . . . . This we finally decided was due to a species of *Fusarium*. . . . . The growers at Santa Cruz moved out to the Santa Barbara district shortly after, so that no more work has been done on the problem."

The occurrence of this disease in other states has been definitely established from diseased freesia corms received from California, Florida, and Indiana. It is probably present also in every other state where the plants are grown extensively for the market.

LOSSES.—Some evidence of the heavy losses caused by this wilt and corm rot was obtained in counts of infected plants and corms in the field, and in inspection of commercial corms in the laboratory at College Station. During 1927, 85 per cent of the plants in several plantings of freesias at Donna, Texas, were destroyed outright by the wilt, and the corms from the remaining apparently healthy plants were already partially decayed. Of some 2000 corms from an infested field at Donna, only 13 per cent were apparently normal, 72 per cent had definite but as yet small lesions, and 15 per cent showed advanced decay.

Of a shipment received from California on November 30, 1931, containing 450 corms, 18 per cent were apparently normal, 75 per cent showed definite early outside lesions, 5 per cent showed an advanced stage of core rot, and 1 per cent were completely decayed. Other shipments from California were badly affected with corm rot also. Corms received from Florida and from Indiana were in no better condition. A statement from a Florida grower is of interest: "In going over my freesias it is almost impossible to find those that have no indication of trouble. There is a discoloration on all of them. . . . . The freesias from the Pacific Coast are infected also."

[4] In a letter dated October 18, 1927.



FIGS. 1, 2.—Corm rot of freesias: fig. 1, longitudinal sections of sprouting corms showing infection spreading from center through vascular system and girdling base of new growth; fig. 2, *Fusarium* growing out from portions of infected corms, in petri-dish culture.

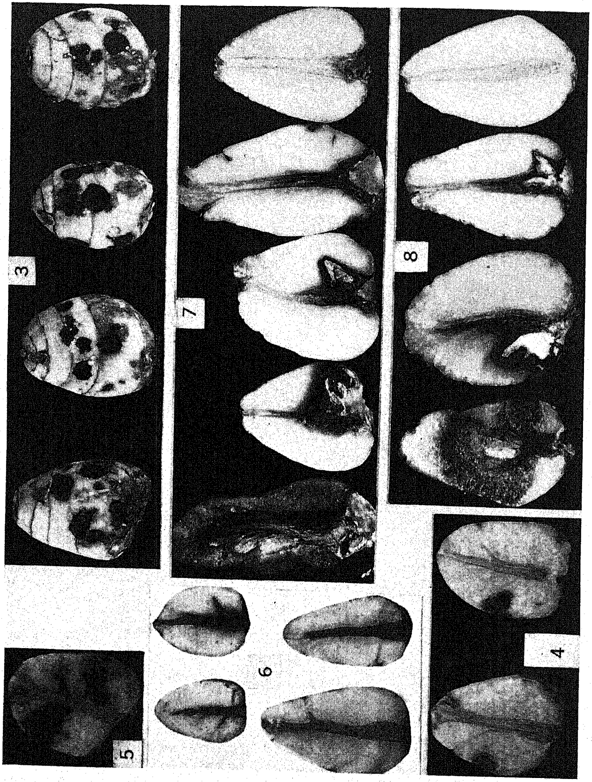
Description of the disease

The following description is based on observations of the disease under field conditions at Donna, Texas, and additional observations of diseased corms received from California, Florida, and Indiana.

In the field the disease usually appears in spots before the plants bloom and continues to spread until time of harvest. The scattered infested areas throughout the field coalesce eventually into large dead spots 40 to 50 feet or more in diameter. At first a few plants appear yellowish, then gradually wilt, die, and collapse on the ground (fig. 25). With collapse of the affected plants, the yellow color of the leaves disappears completely and the dead foliage takes on a straw to papery-white color. The dead leaves gradually decay as they lie on the ground and in time disappear. Plants adjoining infected ones on either side are still erect and normal, but in a few days they too gradually take on the characteristic yellow color, collapse, and die.

The disease attacks not only the tops, but also spreads into the main roots, the larger laterals, and the feeding rootlets, all of which take on a typical pink color. These roots finally decay and assume a dark brown color which in a short time becomes obscured by a superficial coating of the mycelium and spores of a *Fusarium* sp. So far as noticed, infected roots do not soften but remain firm and practically intact although discolored and dry. Infection apparently occurs near the crown of the plant. The top may die only after the disease has invaded the underground parts and then spread into the stem; or the top may die as an initial result of infection before the disease has extended into the corms.

From the infected and dead roots the disease spreads to the corms, which may be found in various stages of infection. With relatively mild infection, the husks may conceal the few or many superficial to deep pinkish lesions on the outside of the corms (figs. 3, 4, 5). These lesions may be the result either of infection from the outside, spreading into the corm, or of infection from the inside spreading outward. In the latter case the heart or interior of the corm is invariably discolored and decayed, although firm (fig. 6). Corms with definite internal decay may usually be recognized by the



FIGS. 3–8.—Corm rot of freesias: fig. 3, corms with husks removed to show external lesions; figs. 4–8, longitudinal sections of infected corms: 4, with external lesions; 5, with more advanced decay arising from external lesions; 6, with decay confined to core and starting from plate of corm; 7, various stages of corm rot; 8, normal corm at right, cracking characteristic of advanced decay at left, and intermediate stages between.

fact that the central root clings to the plate of the corm, while the roots pull out readily from a normal corm and leave a clean scar.

The interior of a normal corm is white and of a mealy appearance. In diseased corms, however, the tissue becomes first yellowish and then, as decay progresses, there appears a definite dark brown discoloration confined at first to the center of the corm. We have designated this type of injury as "core rot." From the center the disease spreads through the ramifications of the vascular system (figs. 1, 6). In well advanced stages the browned vascular system is usually surrounded by a pink discoloration. Occasionally some yellow color also is found in the more advanced decayed tissue in the heart of the corm. In advanced stages of core rot, the center of the corm often cracks and deep cavities form which become filled with an almost pure growth of a *Fusarium* sp. (figs. 7, 8). As decay spreads and involves the entire corm, the affected tissue becomes of uniform chalky-white color, and of a hard, dry, friable, gritty texture, crumbling readily under light pressure. The writers have used the term "chalky dry rot" for this type of infection. The chalky type of dry rot occurs not only as the final stage of decay of entire corms, but also in small pockets arising from initial external infections.

Cause of the disease

From the beginning of these studies it appeared evident that the cause of the trouble was probably a *Fusarium* sp., since this organism was nearly always found on the surfaces of dead roots, on badly decayed corms, and in cracks in the decayed corms. Isolations were made from surface-sterilized blocks of infected tissue of freesia corms grown respectively in Texas, California, Florida, and Indiana (fig. 2). As indicated in table I, the organisms obtained almost without exception were colonies of *Fusarium* spp., with only occasional occurrence of *Penicillium*, *Rhizoctonia*, *Verticillium*, or *Trichoderma*.

Corms for inoculation purposes were received from Indiana, California, and Florida. They were carefully examined, and it was found necessary to cull out large numbers of diseased corms before enough passably normal ones could be obtained for inoculation purposes. Inoculations were made with four *Fusarium* species isolated repeatedly from the infected corms from Texas, California,

Florida, and Indiana, and identified by Dr. C. D. SHERBAKOFF as *F. solani*, *F. bulbigenum*, *F. moniliforme*, and *F. martii-minus* respec-

TABLE I

ISOLATION CULTURES FROM DIFFERENT STAGES OF FREESIA CORM ROT (VARIETIES PURITY AND FLORAL TREASURE)

SOURCE OF MATERIAL AND DATE OF CULTURES	STAGE OF DECAY	NO. OF PIECES OF INOCULUM	RESULTS AFTER 7–14 DAYS: NO. OF FUSARIUM COLONIES	RESULTS AFTER 7–14 DAYS: NO. OF COLONIES OF OTHER ORGANISMS*
Donna, Texas, May 1, 1929	Early core rot	95	73	3 R, 5 P
	Medium core rot	77	64	12 P, 7 V
	Advanced chalk rot	191	125	10 P, 1 T
Donna, Texas, May 7, 1929	Superficial lesions	125	92	0
	Chalk pockets	200	163	12 P
	Early core rot	175	114	22 P
	Advanced core rot	125	120	19 P
	Advanced chalk rot	180	112	0
Indianapolis, Indiana, Sept. 10, 1929	Superficial lesions	250	117	0
	Early core rot	100	81	11 P
	Advanced core rot	125	107	9 P
	Advanced chalk rot	105	84	14 P
Donna, Texas, June 3, 1930	Superficial lesions	100	96	5 R
	Early core rot	100	105	9 P
	Advanced core rot	90	78	7 P
	Advanced chalk rot	70	63	12 P
Costa Mesa, California, April 14, 1930	Superficial lesions	175	99	3 P
	Chalk pockets	175	133	0
	Early core rot	70	63	0
	Advanced core rot	95	87	3 P
	Advanced chalk rot	60	52	0
Carlsbad, California, Nov. 5, 1930	Superficial lesions	200	173	0
	Early core rot	200	187	0
	Advanced core rot	100	98	0
	Advanced chalk rot	100	100	0
Fern Park, Florida, Oct. 28, 1930	Superficial lesions	140	125	0
	Chalk pockets	126	106	14 P
	Early core rot	100	97	3 P
	Advanced core rot	75	62	1 P
	Advanced chalk rot	105	69	4 P

* P, *Penicillium*; R, *Rhizoctonia*; T, *Trichoderma*; V, *Verticillium*.

tively. Additional species tested were *F. batatatis* from sweet potatoes, *F. lycopersici* from tomatoes, *F. conglutinans* from cabbage, *F.*

niveum from watermelons, *Fusarium* sp. from Panama wilt of bananas (all furnished by Dr. SHERBAKOFF); and *F. vasinfectum* from cotton, and a *Fusarium* sp. from decayed gladiolus corms.

All corms used in these tests were first disinfected for 5 minutes in 1–2000 bichloride of mercury in 25 per cent alcohol, then rinsed in sterilized water and thereafter handled aseptically. Inoculations were made by three different methods. In one series, pure cultures of the organisms, grown on whole freesia corms in flasks, were mixed with soil previously steam-sterilized. Four 5-inch pots were used for each culture, and five normal corms were then planted in each pot of inoculated soil. In another series, some selected corms were inoculated with bits of pure culture inserted in punctures made with a sterile needle; and other corms were inoculated by dipping them into suspensions of spores from the pure cultures. The inoculated corms were incubated in large dry chambers at room temperature. Non-inoculated checks were used for each series.

It is to be noted from table II that all the *Fusarium* species isolated from freesias, and in addition *F. conglutinans* and *F. lycopersici*, caused typical damping-off of the plants as well as completely destroying the new roots and causing typical corm rot (figs. 15–23). *F. vasinfectum* caused typical corm rot but did not kill the plants. The other three species of *Fusarium* tested, *F. batatatis*, *F. niveum*, and the species from gladiolus, produced only a decay of the plate of the corm and of the main rootlets, without destroying the top of the plant. In direct puncture inoculations of the corms in moist chambers, however, it was found (table III) that all the species tested could cause typical core rot. Some superficial lesions were observed even on the check, uninoculated corms, but this was not surprising since the corms used in the experiments were merely the less obviously diseased ones selected from lots in which considerable natural infection was present. Considering the very much larger percentages of infection secured with all the *Fusarium* inoculations, however, there can be little doubt but that these fungi were actually responsible for the typical damping-off and corm rot symptoms produced on the inoculated plants. The organisms used in inoculation were finally recovered from the artificially inoculated plants (figs. 9–14).

It appears of special interest that a number of species of *Fusarium*,

in addition to the four isolated originally from freesia corms, have proved definitely pathogenic to freesias. Freesia corms apparently offer an exceptionally favorable medium for species of *Fusarium*,

TABLE II

RESULTS OF PLANTING FREESIAS IN STERILIZED SOIL INOCULATED WITH VARIOUS FUSARIUM SPECIES. SOIL INOCULATED DEC. 17, 1930; CORMS PLANTED JAN. 15, 1931; FINAL NOTES TAKEN FEB. 20, 1931

INOCULUM	PERCENTAGE INFECTION	EFFECTS OF DISEASE		
		MOTHER CORMS	NEW ROOTS	TOP GROWTH
Fusaria isolated originally from diseased freesia corms:				
F. bulbigenum from corms from				
Donna, Texas.........	100	Typical core rot	Complete decay	Dwarfing, then damping-off
Carlsbad, Calif.........	100	" " "	" "	" " " "
Fern Park, Fla.........	100	" " "	" "	" " " "
F. martii-minus from corms from				
Indianapolis, Ind.......	100	" " "	" "	" " " "
Donna, Texas.........	100	" " "	" "	" " " "
Costa Mesa, Calif......	100	" " "	" "	" " " "
Fern Park, Fla.........	100	" " "	" "	" " " "
F. moniliforme from corms from				
Donna, Texas.........	100	" " "	" "	" " " "
Fern Park, Fla.........	100	" " "	" "	" " " "
F. solani from corms from				
Costa Mesa, Calif......	100	" " "	" "	" " " "
Indianapolis, Ind.......	100	" " "	" "	" " " "
Donna, Texas.........	100	" " "	" "	" " " "
F. batatatis from decayed sweet potatoes..........	100	Decay of plate of corms only	" "	Dwarfing, trace of damping-off
F. conglutinans from wilt-infected cabbage plants....	100	Typical core rot	" "	Dwarfing and damping-off
F. lycopersici from wilt-infected tomato plants.....	100	" " "	" "	" " " "
F. niveum from wilt-infected watermelon plants.......	100	Decay of plate of corms only	" "	Dwarfing only
F. sp. from decayed gladiolus corms from Calif........	100	Decay of plate of corms only	" "	Dwarfing, trace of damping-off
F. vasinfectum from infected cotton plants from Brazos Co., Texas..............	100	Typical core rot	" "	Dwarfing, trace of damping-off
None, check..............	0	Normal	Normal	Normal
" "	0	"	"	"

and this is no doubt the cause of the widespread occurrence of the wilt and corm rot. It may be anticipated that further study will demonstrate that many more species of *Fusarium* are capable of causing decay of freesias.

It is not uncommon to find the corms, in various stages of natural decay, covered with various other fungi, notably *Trichoderma*, *Penicillium*, and *Rhizoctonia*. These three organisms were inoculated on

TABLE III

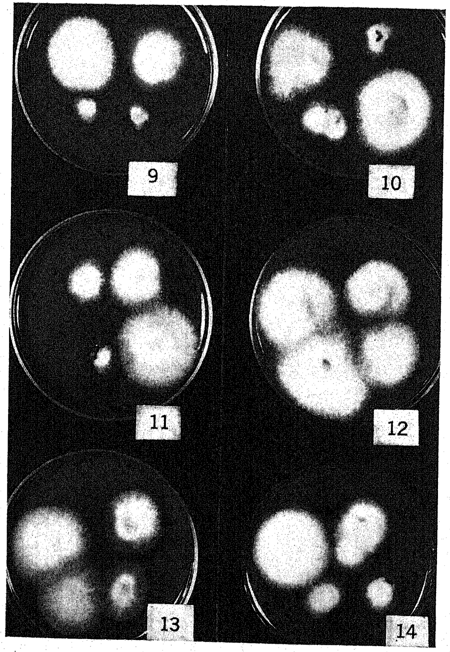
INOCULATION OF INDIVIDUAL FREESIA CORMS (VARIETY SPLENDENS) WITH FUSARIUM SPP., 75 CORMS PER SERIES, IN LARGE DRY PETRI DISHES KEPT AT ROOM TEMPERATURE; NOVEMBER 20–DECEMBER 23, 1930

Inoculum	Method of inoculation	Results after one month	
		Percentage corms infected	Remarks
Fusaria isolated originally from diseased freesia corms:			
F. bulbigenum from corms from			
Donna, Texas	N†	100	Typical core rot
Fern Park, Fla.	N	100	" " "
" " "	S	100	*Superficial lesions
Carlsbad, Calif.	S	100	" "
F. martii-minus from corms from			
Donna, Texas	N	100	Typical core rot
Carlsbad, Calif.	N	100	" " "
" "	S	100	Superficial lesions
Fern Park, Fla.	S	100	" "
F. moniliforme from corms from			
Donna, Texas	N	100	Typical core rot
Fern Park, Fla.	N	100	" " "
" " "	S	100	Superficial lesions
Carlsbad, Calif.	S	100	" "
F. solani from corms from			
Donna, Texas	N	100	Typical core rot
Costa Mesa, Calif.	N	100	" " "
" " "	S	100	Superficial lesions
F. batatatis from sweet potato plants	N	58	Typical core rot
	S	60	Superficial lesions
F. lycopersici from tomato plants	N	75	Typical core rot
	S	40	Superficial lesions
F. niveum from watermelon plants	N	50	Typical core rot
	S	60	Superficial lesions
F. sp. from Panama wilt of bananas	N	90	Typical core rot
	S	82	Superficial lesions
F. vasinfectum from cotton plants	N	86	Typical core rot
	S	80	Superficial lesions
None, check	N	0	Normal
	N	Trace	Superficial lesions

* Superficial lesions resulting from surface inoculations were typical but less than 2 mm. in diameter.
† N, inoculation through needle punctures; S, inoculation by dipping corms in suspension of spores.

normal corms, but the moist decay produced was unlike that caused by the *Fusarium* spp.

It seemed of interest to determine whether the *Fusarium* species which cause freesia core rot might also be able to infect other bulbs.



FIGS. 9–14.—Reisolation of *Fusarium* spp. from inoculated freesia plants shown in figs. 16–21.

One inoculation experiment was run with Bermuda onions, which were washed in tap water, dipped for 10 minutes in 1–2000 $HgCl_2$ in 25 per cent alcohol, and then rinsed twice in sterilized water. The

inoculum was placed in slits in the two outer scales, in three different places on each bulb. As shown in table IV, of the four species tested, only *Fusarium solani* and *F. martii-minus* were able to cause decay of inoculated onion bulbs. The symptoms observed were totally unlike those caused on inoculated freesia corms. *F. solani* and *F. martii-minus* were recovered from the respective inoculated onion bulbs.

TABLE IV

INOCULATION OF ONION BULBS WITH FUSARIUM SPP. ISOLATED ORIGINALLY FROM DISEASED FREESIA CORMS; 15 BULBS PER SERIES. INOCULATED FEB. 26, 1931; FINAL NOTES TAKEN MARCH 26, 1931; BULBS KEPT IN DRY CLOSED CHAMBERS

Inoculum	Percentage infection	Effect on onion bulbs
F. bulbigenum from corms from Donna, Texas	0	All bulbs sound
F. martii-minus from corms from Carlsbad, Calif.	100	Moist but solid decay, involving $\frac{1}{3}$ of bulb
F. moniliforme from corms from Carlsbad, Calif.	0	All bulbs sound
F. solani from corms from Donna, Texas	100	Moist but solid decay, involving $\frac{1}{3}$ of bulb
Check, uninoculated	0	All bulbs sound

In another experiment, normal gladiolus corms were planted in soils previously heavily inoculated respectively with *F. solani*, *F. bulbigenum*, *F. martii-minus*, and *F. moniliforme*, all isolated from infected freesia corms. With all four species of *Fusarium*, the plates of the corms were attacked by a decay which in no case penetrated deeply into the interior of the corms. This localized infection, however, prevented development of new roots from the diseased plate. It would therefore appear unsafe to plant gladiolus in soil infested with the *Fusarium* species which cause freesia corm rot.

Soil, soil screenings, and freesia corms as disease carriers

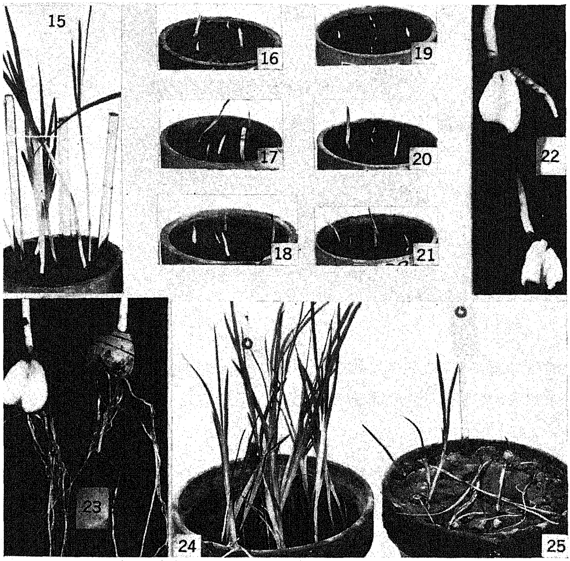
A quantity of infested soil was secured from an infested field at Donna, Texas. The soil, a typical Victoria clay loam, was dug out to a depth of 8 inches and shipped in sacks to College Station in August, 1930. Upon arrival, some 48 hours after shipment, it was carefully screened through a quarter-inch sieve to remove pieces of infected freesia roots and corms. The screenings were saved for an-

other experiment to be mentioned presently. The sifted soil was then placed in 50 5-inch pots, of which 25 were steam-sterilized for 4 hours at 10 pounds' pressure and the remainder left unsterilized. On September 5, 1930, both series of pots were planted with disinfected, apparently normal corms, about ten corms to each pot. Growth started apparently normally in both series; but after 5 weeks the plants in the unsterilized soil began to show typical symptoms of yellowing and damping-off, while none of the plants in the steam-sterilized soil appeared to be attacked (figs. 24, 25). After 3 months the plants were removed from the soil for final examination. Those from the steam-sterilized soil showed only occasional slight infection on the roots and on the exterior of the parent corms themselves. This infection was presumably carried with the parent corms. On the other hand, 80 per cent of the plants from the unsterilized soil had succumbed to typical damping-off, and typical core rot was found on the new corms which were produced in these pots.

It was of interest to determine further whether a soil, virgin as to freesia culture, would also carry the infection. Accordingly, Lufkin fine sandy loam material was secured from one of our cotton plats at College Station. The soil was carefully sifted to remove roots and other trash, and the sifted soil material was placed in 24 5-inch pots, of which half were steam-sterilized. Both series of pots were then planted with normal freesia corms. Not a single case of corm rot or wilt appeared on the plants which grew in the sterilized soil, while definite lesions and discolorations were found on the new corms in the unsterilized soil. However, the typical yellowing and damping-off was absent. This mild infection was apparently caused by organisms occurring in the virgin soil.

In California and other freesia-growing areas, the growers have noticed that continued growing of freesias in infested soil results in heavier losses from the disease. A California grower, for instance, writes that freesias planted on new land where nothing had been grown for at least seven years suffered only 5–10 per cent loss, while at least 75 per cent of the crop died out on old land which had been planted with freesias for the two preceding years. The small percentage of disease on the new land was probably due to introduction of the disease with the corms which were used for planting or to the various other *Fusarium* species already present in the soil.

It has been stated previously that screenings of infected and dead freesia roots and corms were removed from the soil secured from Donna. These screenings were mixed with steam-sterilized soil,



FIGS. 15–25.—Inoculation experiments with freesias: fig. 15, normal check plant; figs. 16–21, dwarfing resulting from inoculation with various *Fusarium* spp.; fig. 22, longitudinal section of typical dwarfed plants showing early stage of corm decay and destruction of all roots; fig. 23, longitudinal section and exterior view of normal corms and roots from check plants shown in fig. 15; fig. 24, normal plants growing in steam-sterilized soil from infested freesia field; fig. 25, damping-off type of injury resulting from planting of normal corms in soil from same source.

placed in ten 5-inch pots, and planted with disinfected, apparently sound, freesia corms. As checks, apparently normal corms were planted in ten 5-inch pots of sterilized soil. At the end of the experi-

ment, 90 per cent of the plants in the pots with the screenings from the infested freesia soil had succumbed to typical damping-off and core rot. In the check pots, only a trace of the plants showed exterior symptoms of core rot lesions.

Fifty freesia corms with superficial lesions were selected at random from the crop of an infested field at Donna, Texas. The husks were removed and the corms washed in tap water, disinfected for 5 minutes in 1–2000 $HgCl_2$ in 25 per cent alcohol, and rinsed twice in sterilized water. These disinfected corms were then planted on September 8, 1930, ten corms to each of five 8-inch pots of steam-sterilized soil. All of the corms germinated apparently normally; but after 18 days, the plants in all the pots ceased growing and gradually lost the normal green color. In 12 more days, the plants in all five pots had wilted and died. The symptoms were similar to those observed on plants in the field. A check planting of 50 apparently normal corms from Indiana remained healthy with the exception of two plants.

Summary

1. *Fusarium* wilt and corm rot of freesias is prevalent in California, Florida, and Indiana, and has become destructive in Texas with the introduction of extensive freesia growing. Four species of *Fusarium*, *F. bulbigenum*, *F. martii-minus*, *F. moniliforme*, and *F. solani*, were isolated from infected freesia corms, and reproduced the disease in inoculation experiments. *F. conglutinans* from cabbage plants and *F. lycopersici* from tomato plants also induced typical symptoms on inoculated freesias; and five other *Fusarium* species were found able to cause somewhat less severe injury. It is probable that other species of *Fusarium* may at times cause wilt and corm rot of freesias. The disease is carried by infected corms, by old remnants of infected plants, and by the soil.

2. *Fusarium solani* and *F. martii-minus* caused decay of inoculated onion bulbs, but the other two *Fusarium* spp. isolated from freesia corms did not attack onions. Normal gladiolus corms planted in soil inoculated with the four species of *Fusarium* from freesias were attacked by a decay of the plates, which also prevented development of new roots.

Agricultural Experiment Station
College Station, Texas

MACROSPOROGENESIS AND EMBRYOLOGY OF MELILOTUS[1]

D. C. COOPER

(WITH PLATES II, III)

Introduction

Perhaps the earliest detailed study of the embryo sac of *Melilotus* is that by GUIGNARD (7, 8), who described the embryo sacs of a number of the Leguminosae. YOUNG (16) figured a series of stages in the development of the embryo sac and embryo of *M. alba*. In the short paper accompanying the plates he reported that "in *Melilotus* the macrospore mother cell develops into the embryo sac without first undergoing tetrad division." CASTETTER (2) gave a detailed account of microsporogenesis and of the development of the pollen grain. He was especially interested in the "furrowing" process in the formation of the microspores. SMITH (12) confirmed the chromosome count as given by CASTETTER. ELDERS (5), FRYER (6), and CLARKE (3) have counted the chromosomes in the root tips of a number of species of *Melilotus* and have found the same diploid number, 16, in all the species examined.

Materials and methods

Material from two species of sweet clover, *Melilotus alba* and *M. officinalis*, and from the variety Redfield Yellow (which is possibly a derivative of a hybrid between those two species),[2] was used in this investigation. Buds, open flowers, and young fruits were taken from plants growing in the Genetics greenhouses at the University of Wisconsin during the late winter and early spring of 1932. These were fixed in La Cour's chromo-acetic-osmic fixative, in Licent's solu-

[1] Papers from the Department of Botany and the Department of Genetics (no. 150), Agricultural Experiment Station, University of Wisconsin. Published with the approval of the Director of the Station.

[2] Unpublished results of investigations by R. A. BRINK. Acknowledgment is due Mr. L. W. KEPHART, Division of Forage Crops and Diseases, U.S. Department of Agriculture, Washington, D.C., for furnishing the Redfield Yellow plants used from the Department stock at Redfield, S.D.

tion (80 cc. 2 % chromic acid, 5 cc. glacial acetic acid, 15 cc. commercial formalin), in Flemming's medium solution, and in Karpechenko's modification of Nawaschin's fluid. Since the young buds tend to float on the surface of an aqueous solution, they were first dropped into Carnoy's alcohol-acetic-chloroform fluid for a short time (10–20 seconds) and then transferred to the fixative, in which they immediately sank. The older flowers were dissected, and the pistils removed and fixed separately. For stages in fertilization, pistils were fixed at periods 18, 21, 27, 32, 36, 40, and 45 hours respectively after pollination. Karpechenko's modification of Nawaschin's fluid and Licent's solution gave the best results with the embryo sacs and with stages in embryo development. In the latter fluid the endosperm was particularly well fixed.

After imbedding in paraffin, sections were cut 8–12 μ in thickness, mounted serially, and stained in Haidenhain's iron-alum haematoxylin, Ehrlich's haematoxylin, or a combination of the latter with light green.

No significant differences were found between the species studied so far as concerns the chromosome number or the history of the macrospore mother cell, the macrospores, and the embryo sac. Since the material for the study of fertilization and embryo development was limited to the variety Redfield Yellow, the description which follows is based chiefly upon the work with that variety.

Investigation

DEVELOPMENT OF OVULE.—The ovules arise in two rows, one on each side of the ventral suture of the ovary. There are usually two ovules in each row, which are spaced far enough apart so that they alternate with those of the adjoining row. As many as six ovules have been found in an ovary, but the usual number is four. The young ovule is initiated by the multiplication of the hypodermal cells of the placenta. Protuberances are thus pushed out into the cavity of the ovary, and, as these continue to develop, cell division becomes more active on one side than on the other, with the result that the ovule bends toward the base of the ovary. REEVES (10) found that in *Medicago* the direction of this curvature may be determined mechanically by the growth of the carpel. The young ovule

is orthotropous, according to him, until it comes in contact with the dorsal wall of the ovary; then it begins to bend, usually toward the base. In *Melilotus* the curvature of the ovule begins before its nucellus touches the dorsal wall of the ovary, and no part of the ovule is in contact with the dorsal wall until it is almost mature.

The ovules usually curve toward the base of the ovary, although in a few instances it has been noted that the apical ovule curved toward the stylar end of the ovary. This is similar to the condition in *Medicago* (**10**) and very different from that in *Phaseolus* (**15**), where all the ovules curve toward the apex of the ovary.

The inner integument arises from the epidermis of the ovule at a level just basal to the macrospore mother cell, at about the time that that cell is in an early heterotypic prophase stage (fig. 4). Shortly thereafter the outer integument arises from the epidermis at a level just below the inner integument (figs. 5, 6). Both integuments, each composed of two layers of cells, develop so as nearly to cover the nucellus. The outer integument grows more rapidly than the inner one and reaches a level almost even with the apex of the nucellus by the time the four macrospores are formed (fig. 11). The outer integument continues to grow more rapidly on that side of the ovule away from the placenta, and the curvature of the ovule continues so that the micropyle lies parallel to the funiculus while the ovule is still at right angles to the latter and the inner integument has just reached the level of the apex of the nucellus (fig. 15). The ovule is typically campylotropous by the time the embryo sac is mature (fig. 17). At that stage the outer integument is massive in structure in the region of the micropyle, whereas the inner integument is only two cell layers in thickness and has grown over the apical end of the embryo sac so as to leave a very short inner portion of the micropyle.

At an early stage in the development of the ovule, and at about the time that it begins to curve, a hypodermal cell at the apex of the nucellus becomes differentiated as an archesporial cell (fig. 1). This cell divides to form an inner sporogenous and an outer parietal cell (figs. 2, 3). The outer parietal cell divides further, forming additional nucellar cells. It is usually divided longitudinally only, a single layer of parietal cells thus being produced (figs. 4, 5). Occasionally it divides once transversely (fig. 7) and then longitudinally, so that two

layers of parietal cells are formed. The sporogenous cells of *Melilotus* are not so deeply imbedded in the nucellus as is the case in other Leguminosae studied by GUIGNARD (7, 8), MARTIN (9), and REEVES (10).

The primary sporogenous cell is easily recognized by its greater size (fig. 3), by the greater size of its nucleus, and by its peculiar staining properties. Only one sporogenous cell appears in the nucellus of the Redfield Yellow variety and in *M. officinalis*, although in most instances two or three such cells are found in *M. alba*. These multiple sporogenous cells have much the same appearance as those described by REEVES for *Medicago sativa*.

MACROSPOROGENESIS.—The primary sporogenous cell (fig. 18) becomes the macrospore mother cell without further division. It increases in length as well as in diameter, so that at its greatest size it is approximately twice as long as wide. In the early heterotypic prophase the nucleus of the macrospore mother cell contains one conspicuous nucleolus imbedded in the chromatic network. This network condenses toward one side of the nucleus to form a dense synizetic knot (fig. 19). On recovery from synizesis, loops of paired threads spread out toward the nuclear membrane (fig. 20) until the open spireme stage is reached. Transverse segmentations of the open spireme form the chromosomes, which early appear as pairs of threads (fig. 21). These shorten and thicken until a typical diakinesis stage is reached wherein 8 pairs of chromosomes can be counted (figs. 22, 24).

The chromosomes of *Melilotus*, as seen on the equatorial plate of the heterotypic division, are very similar in size and shape. Eight pairs can be distinguished in both metaphases and anaphases (figs. 25, 26).

After the heterotypic division, the macrospore mother cell is divided by means of a cell plate in such a manner that the basal cell is somewhat larger than the apical one. This cell division is not directly transverse but is at an oblique angle to the longitudinal axis of the cell (fig. 9). After the homoeotypic division a row of four macrospores is formed. The plane of the second cell division is at an oblique angle to that of the first, so that the macrospores may not appear to be in a linear row (figs. 10, 27). The formation of four macro-

spores was also observed in *Melilotus alba* (fig. 12) and *M. officinalis* (fig. 13). GUIGNARD (7, 8) observed the formation of only three macrospores in *Phaseolus multiflorus* and *Medicago arborea*. WEINSTEIN (15) likewise found that only three macrospores are formed in *Phaseolus vulgaris*. Most observers of the Leguminosae, however, have found a row of four macrospores. There is some variation as to the position of the functional macrospore. GUIGNARD found that the third macrospore from the micropylar end of the tetrad functions in several species of *Acacia*, and SAXTON (11) found a similar situation in *Cassia tomentosa*. In *Melilotus*, as in the majority of the Leguminosae thus far examined, however, it is the chalazal macrospore that functions in the formation of the embryo sac.

CHROMOSOME NUMBER.—CASTETTER (2) reported the haploid number of chromosomes of *Melilotus alba* as 8. This count was verified by SMITH (12). ELDERS (5) counted the chromosomes in the root tip cells of three species, *M. alba*, *M. alba annua*, and *M. officinalis*, and found the diploid number to be 16. FRYER (6) reported the diploid number as 16 in *M. sulcatus* and *M. indica*, as well as in those species already studied. He found that one pair of the chromosome complement of *M. alba* is distinguished by the presence of satellites. CLARKE (3) also found 16 to be the diploid number of chromosomes in *M. wolgica*, *M. italica*, *M. segetalis*, and *M. messanensis*.

The haploid number of chromosomes is 8 in Redfield Yellow as well as in *M. alba* and *M. officinalis*. These counts were made in the microspore mother cells and in the macrospore mother cells of Redfield Yellow. At early diakinesis a pair of satellite chromosomes is present in this variety (fig. 22). In late diakinesis, and on the heterotypic equatorial plate, the chromosomes are more or less rounded and the satellites are not distinguishable (figs. 23, 25). A polar view of an equatorial plate stage of a nucleus of the endosperm shows 24 chromosomes. Satellites have been seen attached to two of these chromosomes (fig. 41).

DEVELOPMENT OF EMBRYO SAC.—The chalazal macrospore functions as the embryo sac mother cell, and the other three macrospores disintegrate (fig. 27). By three nuclear divisions an 8-nucleate cell is formed (figs. 14, 15, 28). Cell walls cut off three small cells, each

with a single nucleus, at either end of the embryo sac, leaving one large middle cell with a nucleus near each end, the central region being highly vacuolate (fig. 16). During this time the parietal cells surrounding the embryo sac are disintegrating, so that it ultimately comes to lie in contact with the epidermal cells of the nucellus.

These observations are at variance with those of YOUNG (16), who reported the "lily" type of macrogametophyte development for *M. alba*. In preparations of both *M. alba* and *M. officinalis*, as well as in Redfield Yellow, a row of four macrospores was characteristic (figs. 11, 13). The embryo sac in each case develops from the chalazal macrospore and the other macrospores disintegrate. A comparison of figure 14, showing a 2-nucleate macrogametophyte, with YOUNG's figure 15 would indicate that they are at approximately the same stage of development. In both figures disintegrating nucellar cells are present, whereas at the time of the heterotypic and homoeotypic divisions (figs. 7, 10) the nucellar cells have not yet begun to break down.

After the formation of the 7-celled embryo sac, a considerable time elapses during which the ovule develops rapidly and the embryo sac elongates until it is several times as long as wide (fig. 17). In this process the epidermal cells of the nucellus in the micropylar region break apart and the embryo sac grows out so that ultimately more than a third of it is in direct contact with the cells of the inner integument. WEINSTEIN found, in *Phaseolus*, that some of the nucellar cells form a cap or protecting layer over the apical portion of the embryo sac. Such a layer is not present in *Melilotus*.

The cells of the nucellus at the chalazal end of the embryo sac are broken down and digested in the process of elongation, so that the basal portion of the embryo sac becomes deeply imbedded. The continued curvature of the ovule causes the embryo sac to be bent at an angle in its middle region. The antipodals usually disintegrate before fertilization occurs. GUIGNARD (7) found that the antipodals of the same species of *Melilotus* may disintegrate early in some embryo sacs and persist until after fertilization in others. The basal polar nucleus moves toward the micropylar end in the denser cytoplasm along one side of the large central vacuole, and comes to lie in close contact with the apical polar nucleus in a region close to the egg apparatus

(fig. 29). The polar nuclei fuse to form the primary endosperm nucleus before fertilization (fig. 30).

The egg apparatus consists of three elongated pear-shaped cells, the egg being somewhat larger than the two synergids. In each synergid a large vacuole appears in the basal region, and the nucleus is imbedded in the dense cytoplasm just above this vacuole and in the middle region of the cell. Just before fertilization, the micropylar ends of the two synergids elongate considerably and extend into the micropyle. The cytoplasm in this region contains minute elongated vacuoles or canals which extend forward from the region of the large basal vacuole (figs. 30, 32). These vacuoles or canals together with dense areas in the cytoplasm give the appearance of a filiform apparatus. This is similar to the condition described by BROWN (1) for *Phaseolus vulgaris* and by the writer (4) for *Lycopersicon esculentum*. A transverse section of the egg apparatus at a level containing the synergid nuclei shows the vacuoles and denser regions in the cytoplasm (fig. 31). There are no dense accumulations in contact with the cell walls and the walls themselves show no peculiar markings.

The broad end of the egg extends beyond the synergids into the embryo sac. There is a large vacuole in the pointed apical region of the egg, and the large egg nucleus is buried in the denser cytoplasm at the base of the cell.

Fertilization takes place between 18 and 21 hours after pollination. In this process a single pollen tube grows down the micropyle and enters the embryo sac between the synergids and the egg. The synergids are not destroyed by the entrance of the pollen tube and may persist until an embryo of some size is formed. WEINSTEIN found that the antipodals and synergids of *Phaseolus vulgaris* disintegrate early, and that at the time of fertilization the embryo sac consists of but two cells, the egg and the primary endosperm cell. This early disintegration of antipodals and synergids has been reported for a number of the Papilionoideae. In *Melilotus*, as already stated, the antipodals usually disintegrate before fertilization, but the synergids persist for some time thereafter. The two male nuclei are discharged from the pollen tube near the egg. Figure 32 shows the micropylar end of an embryo sac at the time of fertilization. One male gamete nucleus is in close proximity to that of the egg and the

other male nucleus is closely appressed to the fused polar nuclei. The male gamete nuclei are not spherical as in *Phaseolus* (15), but are somewhat elongated and a nucleolus is clearly evident near one end of each. Figures 33 and 34 show the process of fusion of a male gamete nucleus with the egg nucleus. Two nucleoli are clearly evident in both figures.

The primary endosperm nucleus divides shortly after fertilization, and a number of nuclei are to be found in the cytoplasm of the embryo sac at the time of the division of the zygote nucleus (fig. 35). YOUNG, MARTIN, and WEINSTEIN report a similar situation in other Leguminosae. On the other hand, STRASBURGER (14) found that the zygote and primary endosperm nuclei divide simultaneously in *Lupinus*. Such a condition was also reported by GUIGNARD for *Phaseolus multiflorus*.

EMBRYO.—The zygote divides transversely (fig. 35) to form a 2-celled proembryo consisting of an enlarged basal cell and a smaller apical cell (fig. 36). The latter cell divides (fig. 37) to form a linear row of three cells (fig. 38). According to SOUÈGES (13), the apical cell of this row by further divisions forms the embryo in *Medicago* and the middle and basal cells divide to form the suspensor. In *Melilotus* a vertical division of the apical cell does not occur until a 4-celled proembryo has been formed (fig. 39). It may be, however, that the two middle cells of this proembryo arose by a division of the middle cell rather than of the apical cell present at the 3-celled stage. The embryo is formed from the apical cell of the 4-celled proembryo only, whereas the multicellular suspensor is formed by vertical and horizontal divisions of the stalk cells of the proembryo (figs. 40, 42). The suspensor persists even after disintegration of the endosperm, and fragments of it can be seen attached to an almost mature embryo (fig. 53).

Figure 39 shows the nuclear division in the apical cell of a 4-celled proembryo. Two further divisions occur, the first vertical (figs. 41*a*, 43*b*) and the second transverse, giving rise to an 8-celled embryo. Periclinal divisions now take place which cut off the dermatogen (figs. 44–47). Multiplication of the cells of the embryo continues, forming at first a spherical mass of cells (figs. 48, 49) which later becomes somewhat pear-shaped (fig. 50), the apical region being broader than the base.

The cotyledons first appear as two outgrowths of the apex of the embryo (figs. 51, 52). These appear simultaneously and are directly opposite each other. They elongate rapidly, forming two elongated, broad, and somewhat flattened structures. These cotyledons at maturity are very broad, flat, leaflike structures that are packed with storage materials.

The basal region likewise develops both laterally and terminally to form the hypocotyl. At the same time a conspicuous knob, the epicotyl, is formed at the apex of the embryo and between the bases of the cotyledons. The embryo curves during the course of its development so that ultimately the cotyledons and hypocotyl come to lie parallel to one another, the most acute part of the curvature being in the apical portion of the hypocotyl in the region of the epicotyl (fig. 53).

ENDOSPERM.—The nuclei of the endosperm are scattered throughout the embryo sac, but the greater number of them are in the region immediately next the embryo. Cell division begins first in that region of the endosperm surrounding the suspensor at about the time of the 16-celled stage of the embryo, and from this time on during the development of the embryo, cell walls are being formed in the region of the embryo and there are free nuclei in the chalazal end of the endosperm (fig. 51). The cell structure of the endosperm is somewhat spongy in character. In ovules containing almost mature embryos no trace of endosperm could be found.

Summary

1. In *Melilotus* a single apical hypodermal cell is differentiated in the young ovule as an archesporial cell. This cell divides to form a primary parietal and a primary sporogenous cell.

2. The primary sporogenous cell functions as the macrospore mother cell in *M. officinalis* and in the Redfield Yellow variety of sweet clover. A number of sporogenous cells (1–3) are usually formed in *M. alba*, but only one of them undergoes further development.

3. In consequence of two divisions, the macrospore mother cell produces a row of four macrospores, the chalazal one of which develops into a 7-celled embryo sac of the usual type; the other three macrospores disintegrate.

4. The micropylar end of the nucellus breaks down and the em-

bryo sac elongates greatly, the basal portion becoming deeply imbedded in the nucellus and the apical portion extending so as to lie in direct contact with the inner integument.

5. The polar nuclei fuse and the fusion nucleus comes to lie just basal to the egg apparatus.

6. The antipodal cells disintegrate just prior to fertilization.

7. The apices of the synergids elongate and a distinct filiform apparatus is produced.

8. The pollen tube enters the embryo sac between the synergids and the egg, and the synergids are rarely broken down in consequence of fertilization but persist for some time thereafter.

9. Fertilization occurs under greenhouse conditions between 18 and 21 hours after pollination.

10. The zygote, by transverse divisions, forms a filament of three or four cells. The terminal cell of this filament forms the embryo. The basal cells by further divisions form a multicellular suspensor.

11. The primary endosperm nucleus divides before the zygote, and there are usually four or more free nuclei in the embryo sac at the time of division of the zygote.

12. Cell division in the endosperm begins in the region of the suspensor at about the time the embryo has reached the 16-celled stage.

13. The endosperm is entirely absorbed by the time the embryo is mature.

14. Remnants of the suspensor are still present after the absorption of the endosperm.

15. The haploid number of chromosomes is 8. *Melilotus alba* and Redfield Yellow each have one pair of satellite chromosomes.

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EXPLANATION OF PLATES II, III

All drawings were made with an Abbé camera lucida at table level. Spencer compensating oculars, a Spencer 8 mm. N.A. 0.60 apochromatic objective, and a Leitz oil-immersion N.A. 1.32 objective were used.

PLATE II

FIG. 1.—Young nucellus showing hypodermal archesporial cell. ×310.

FIG. 2.—Nucellus; archesporial cell divided to form primary sporogenous and primary parietal cell. ×310.

FIG. 3.—Young macrospore mother cell; early prophase. ×310.

FIG. 4.—Ovule showing origin of inner integument. ×310.

FIG. 5.—Ovule showing origin of inner and outer integuments. ×310.

FIG. 6.—Ovule; macrospore mother cell at diakinesis. ×310.

FIG. 7.—Ovule; macrospore mother cell at equatorial-plate stage, heterotypic division. ×310.

FIG. 8.—Somewhat later than fig. 9; heterotypic anaphase. ×310.

FIG. 9.—Daughter cells formed after heterotypic division. ×310.

FIG. 10.—Daughter cells formed after homoeotypic division. ×310.

FIG. 11.—Ovule with linear row of 4 macrospores. ×310.

FIG. 12.—Same stage as fig. 11; from *M. alba*. ×310.

FIG. 13.—Same stage as fig. 11; from *M. officinalis*. ×310.

FIG. 14.—Nucellus with 2-nucleate macrogametophyte; disintegrating macrospores and nucellar cells. ×310.

FIG. 15.—Four-nucleate macrogametophyte. ×310.

FIG. 16.—Nucellus with young 7-celled embryo sac. ×310.

FIG. 17.—Ovule at later stage; 7-celled embryo sac. ×145.

FIG. 18.—Macrospore mother cell; early prophase. ×1060.

FIG. 19.—Synizesis. ×1060.

FIG. 20.—Approaching open spireme stage. ×1060.

FIG. 21.—Nucleus showing chromosome pairs. ×1060.

FIG. 22.—Early diakinesis. ×1060.

FIGS. 23, 24.—Stages in diakinesis. ×1060.

FIG. 25.—Macrospore mother cell with heterotypic equatorial plate. ×1060.

FIG. 26.—Macrospore mother cell; heterotypic anaphase. ×1060.

FIG. 27.—Typical tetrad of macrospores, the three outer spores beginning to disintegrate. ×1060.

FIG. 28.—Embryo sac; divisions leading to 8-nucleate stage. ×625.

FIG. 29.—Apical portion of embryo sac showing micropylar apparatus and fusing polar nuclei. ×310.

FIG. 30.—Same as fig. 29, slightly later stage; polar nuclei fused and synergids beginning to elongate. ×310.

PLATE III

FIG. 31.—Transverse section of synergids and egg in region of synergid nuclei. ×625.

FIG. 32.—Stage in fertilization; one male nucleus closely appressed to primary endosperm nucleus and another in close proximity to that of egg. ×310.

FIG. 33.—Egg nucleus of fig. 32 in detail showing male nucleus closely adjacent. ×1060.

FIG. 34.—Stage in process of fusion of egg nucleus with male nucleus. ×1060.

FIG. 35.—Binucleate proembryo; cell in process of division. ×625.

FIG. 36.—Two-celled proembryo; synergids still present and conspicuous. ×310.

FIG. 37.—Two-celled proembryo; nucleus of apical cell in process of division. ×310.

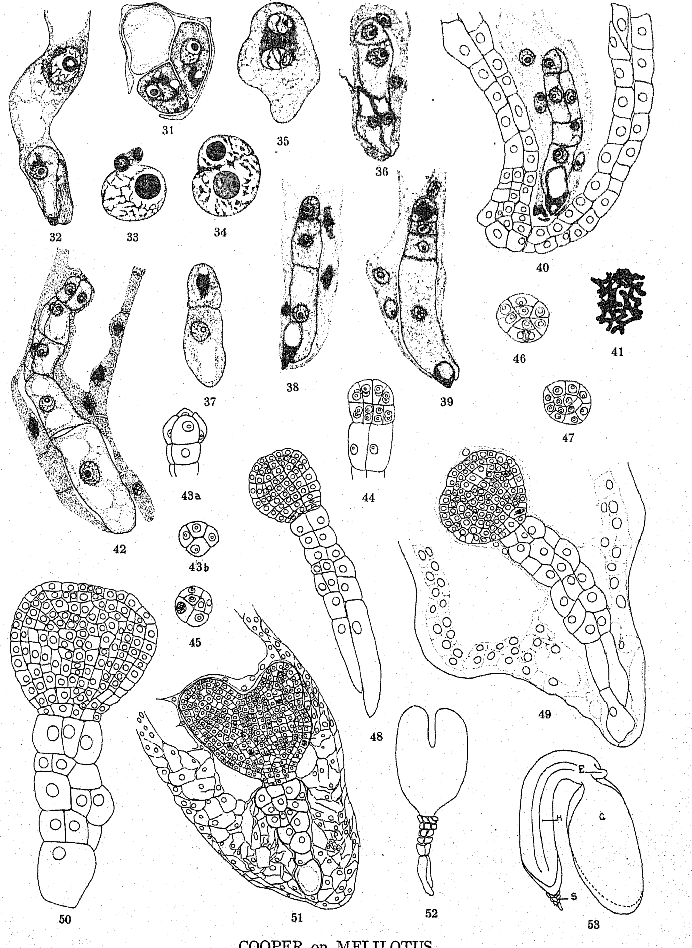
FIG. 38.—Three-celled proembryo; nuclear divisions in endosperm. ×310.

FIG. 39.—Four-celled proembryo; nucleus of apical cell dividing. ×310.

FIG. 40.—Somewhat later stage showing longitudinal division of apical cell and of adjacent suspensor cell. ×310.

PLATE II

COOPER on MELILOTUS



COOPER on MELILOTUS

FIG. 41.—Polar view of equatorial plate in endosperm; 24 chromosomes can be counted. ×1060.

FIG. 42.—Still later stage; further division of suspensor cells; nuclear divisions in endosperm. ×310.

FIG. 43.—Four-celled embryo: *a*, lateral view; *b*, polar view. ×310.

FIG. 44.—Young embryo with 16 cells. ×310.

FIGS. 45–47.—Transverse sections of upper quadrant of embryo showing periclinal divisions. ×310.

FIG. 48.—Older embryo showing well-defined regions and suspensor made up of large cells. ×220.

FIG. 49.—Older embryo, still spherical in shape and with cellular endosperm in the closely adjacent region. ×220.

FIG. 50.—Still older embryo, somewhat flattened apically. ×290.

FIG. 51.—Embryo showing early development of cotyledons; cells formed in the endosperm at apex of embryo; cells of endosperm in region of suspensor loosely arranged. ×145.

FIG. 52.—Embryo showing further development of cotyledons. ×70.

FIG. 53.—Almost mature embryo; remnants of suspensor still present: *c* cotyledons; *e*, epicotyl; *h*, hypocotyl; *s*, suspensor. ×20.

DEVELOPMENT OF THE MALE GAMETOPHYTE IN TRADESCANTIA

KARL SAX AND H. W. EDMONDS

(WITH PLATE IV)

A study of the development of the male gametophyte in *Tradescantia* seems to show that nuclear differentiation is associated with a definite polarity of the microspore, and that in turn this polarity is correlated with the nuclear-cytoplasmic relations at the end of the meiotic divisions. The male gametophyte is favorable for such studies because of the comparatively simple structure, the striking difference in appearance and function of the two nuclei in the mature pollen grain, and the ease with which consecutive stages in development may be obtained.

This investigation was based on aceto-carmine smears of microspore mother cells and microspores at various stages of development. After mounting, the preparation was heated until a few small air bubbles appeared. This treatment results in a better differentiation between nucleus and cytoplasm. After the slide had been heated, it was inverted on a piece of filter paper and slightly pressed to remove the excess fixing fluid, and then sealed with a mixture of gum mastic and paraffin. Such preparations remain in good condition for several weeks, or even months, if kept in a refrigerator. Aceto-carmine smears were also prepared without heating, for examination of the cell granules which are destroyed by heat. Living microspores were also studied as a check on the fixed material. Some material was fixed and sectioned for an examination of cross-sections of microspores.

Investigation

At the first meiotic division the chromosomes pass to opposite sides of the microspore mother cell, and the daughter nuclei are formed near the outer cell wall. Figure 1 is a photograph of a dyad showing the characteristic position of the nuclei. Both of the second meiotic division spindles are usually in the same plane and at right

angles to the plane of the first meiotic spindle. Again the daughter chromosomes pass as far as possible to opposite poles, so that the four nuclei are near the periphery of the tetrad. The nuclei at this stage are shown in figure 2. At the end of the second meiotic division, cell walls are found at right angles to the first, so that the cells of the tetrad are oriented like segments of an orange.

As the tetrad nuclei pass into the resting stage, they take a position near the center of each cell. At a later stage the one-nucleate microspores separate and become independent cells, although they tend to retain the characteristic shape found at the tetrad stage. The one-nucleate microspores, as seen from the side, are ovoid in shape, with one side flattened, this side being undoubtedly the central axis or inner wall of the cells at the tetrad stage. In smear preparations the microspores seldom lie on end so that their morphology in cross-section can be determined, but in sectioned material a cross-section of the microspore across the shorter axis shows an angular outline on one side. As the microspore usually lies on one of the two flattened walls, the flattened side is the angular junction of these two walls, while the outer or rounded side of the microspore is derived from the outer wall of the original microspore mother cell.

In these young microspores a number of refractive granules are especially conpicuous in the cytoplasm. These granules are found at prophase stages of meiosis and persist during the meiotic divisions. In the young microspores they seem to be scattered more or less at random in some cells, although as a rule they are found most abundantly on either side of the nucleus toward the ends of the cell. The granules are shown in figure 3, which is a photograph of a microspore which was not subjected to heat, which would have resulted in disappearance of the granules. As the nucleus enters the prophase stage the granules disappear, and the regions previously occupied by them appear as vacuolate areas.

The nature of these granules has not been determined. They do not seem to be starch grains because they are invisible under a polarizing microscope with crossed Nicolls, and they do not show the typical color reaction with a solution of iodine and potassium iodide. The solidity of the granules is demonstrated by the fact that they are not deformed by pressure on the cover glass, either before

or after extrusion from the cell. On collision they do not coalesce as would fluid droplets.

As the cytoplasmic granules disappear the microspore increases in size, and the flattened side of the cell becomes more conspicuous. It has a smooth wall as contrasted with the corrugated wall of the outer or curved side, and appears to be thicker (figs. 4–6). The nucleus, which at this time is in an early prophase stage, lies nearer the inner wall, at first nearer one end of the cell (fig. 5) but later taking a more central position (figs. 4, 6). During these prophase stages large vacuoles appear in the cytoplasm. In living cells they appear to be spherical, but fixation apparently destroys their smooth, regular outlines. The vacuole or vacuoles may extend from one side of the microspore to the other (fig. 4), but at later stages they are found usually at either side of the nucleus (figs. 6, 8, 9) and separated by the cytoplasm between the nucleus and the outer wall of the cell.

The chromosomes lie flat on the cell plate at the metaphase stage of the microspore division. Three of the chromosomes have approximately median fiber constrictions, while the other three have submedian constrictions (fig. 7). The dividing chromosomes are invariably nearer the inner or flattened wall of the microspore, and the axis of the poles is at right angles to this wall. The vacuoles are conspicuous on both sides of the dividing chromosomes (figs. 8–10).

As the chromosomes pass to the poles, those near the inner or flattened wall of the microspore remain compact, while those near the outer wall may elongate even before a nuclear wall is formed. The compact nucleus is cut off by a thin cell wall which includes very little cytoplasm. The other nucleus increases greatly in size and passes into the resting stage. This is the vegetative or tube nucleus (figs. 9–11). The vegetative nucleus seems to begin disintegration even before the pollen tube is formed, and apparently plays little part in further gametophytic development.

The small or generative nucleus never passes into a typical resting stage. As it enlarges it retains its staining reaction. The thin cell wall surrounding this nucleus is apparently broken as the nucleus rapidly elongates to form a slender sickle-shaped body. The generative nucleus at this stage no longer retains its position near the inner wall of the microspore, but may lie in any region of the cell.

The pollen tube germinates at one end of the microspore. The generative nucleus passes into the pollen tube, where it divides to form the two male gametes. Fertilization occurs about twelve hours after pollination.

Duration of microsporogenesis and gametophyte development

The length of time for development of the different stages in microsporogenesis and gametophytic development has been determined in *Tradescantia reflexa*. The data were obtained by marking

TABLE I

MEIOSIS AND MICROSPORE DEVELOPMENT IN TRADESCANTIA

Day	Microscopic events	Macroscopic events
1–4	P.M.C. prophase	Anthers white
5–6	Meiosis	Anthers pale yellow
7–8	Growth of microspore	Anthers yellow
9–10	Vacuolation of microspore	....
11	Microspore division	Color appears on petals
12	Differentiation of nuclei	....
13	Elongation of generative nucleus	....
14	Mature pollen	....
15	Pollination, A.M. Fertilization, P.M.	Flower opens, A.M. Flower closes, P.M.

the buds with India ink and making smears each day from other buds of the same size from the same plant, the work being done in the field. Considerable variation may be caused by weather conditions. The data are summarized in table I.

In the case of *Tradescantia* plants growing in the field during the summer months, there is evidence of little diurnal periodicity except in opening and closing of the petals. Although division occurs in both the microspores and in the microspore mother cells at all hours of the day or night, it was generally easier to obtain meiotic divisions between 8 and 11 A.M. and microspore divisions between 3 and 6 P.M. The species investigated are self-sterile, and fertilization occurs only when a flower is pollinated with compatible pollen.

Microspore development in partially sterile plants

Several plants of *Tradescantia* have been found which have partially sterile pollen. In one plant (*T. edwardsiana*) the pollen steril-

ity is about 50 per cent, and is caused by non-disjunction of segmental interchange chromosomes (3). More than 80 per cent pollen sterility is found in the related genus *Rhoeo.* In both cases tetrad formation is apparently normal. No differences can be observed in the young one-nucleate microspores, all having the typical granular bodies. At a slightly later stage, however, two classes of microspores are evident. The microspores which develop into normal pollen grains lose their granules as they increase in size, and microspore division occurs. The microspores which do not develop retain the granular bodies and do not increase in size. The nucleus degenerates, and no division occurs. Sterility counts made as soon as these two classes of microspores could be distinguished are essentially the same as those made when the fertile pollen grains are mature. It is evident that chromosome deficiency is associated with failure of microspore development at a very definite growth stage.

Discussion

In the formation of the tetrad in *Tradescantia,* the second divisions are at right angles to the first division, and the division in the microspore is at right angles to the preceding second division. The axis of the microspore division is across the shorter diameter of the cell, in the great majority of cases. At an earlier stage before microspore division, the nucleus lies at one end of the cell surrounded by cytoplasm, while the other end of the cell is vacuolate, with only a peripheral layer of cytoplasm along the cell wall. Before the division occurs the cell becomes bilaterally symmetrical, however, with the nucleus nearer the inner or flattened wall of the microspore. Apparently a definite polarity exists which determines the axis and location of the spindle, although the cytoplasm and nucleus do not become oriented until shortly before division occurs. The polarity of the microspore is presumably determined at the preceding meiotic divisions.

The microspore division occurs in such a way that one cell is formed near the inner wall of the microspore and is cut off with little cytoplasm by a thin wall. This cell does not pass into a typical resting condition but elongates and becomes the generative nucleus. The other nucleus, surrounded by the bulk of the cytoplasm of the

cell, passes into the resting stage and apparently plays no further active part in microspore development. It would appear that the nuclear-cytoplasmic relations might be responsible for the differential development of the two nuclei, but until experimental modification of these relations can be made no definite conclusions are justified. It is clear, however, that nuclear differentiation, in respect to both structure and function, is associated with the position of the nuclei in the microspore.

Development of microspores in partially sterile plants of *Tradescantia* is of interest in respect to the relations between the chromosome complement and development. In this genus a complete haploid set of chromosomes is essential for normal gametophytic development, as is generally the case in plant species. Gametophytes with a haploid complement plus additional chromosomes seem to develop normally, and mature pollen grains are formed. For instance, in a triploid *Tradescantia* nine chromosomes were usually found at the microspore division (1). It is known that one-nucleate microspores with less than six chromosomes are sometimes formed, but none has been found with less than the haploid set at the time of microspore division in *Tradescantia*.

Plants containing segmental interchange chromosomes are usually partially sterile, resulting from non-disjunction, so that each daughter nucleus at the first meiotic division is deficient for a section of a chromosome and duplicate for another segment, although six chromosomes are present. These deficient nuclei in *Tradescantia* and *Rhoeo* are able to divide at the second meiotic division, and one-nucleate microspores are formed, but there is no further development. The nucleus remains in the resting stage and the cytoplasmic granules do not disappear as they do in normal microspores. It is evident that the absence of a chromosome or section of a chromosome prevents normal development of the cell. But why, if a deficient nucleus is able to divide once, does it fail at the time of the next division?

If the chromosome releases some gene product that passes from the nucleus to the cytoplasm with disintegration of the nuclear wall, the cytoplasm at the beginning of the first meiotic division will receive a complete set of genic material. The deficient nuclei formed

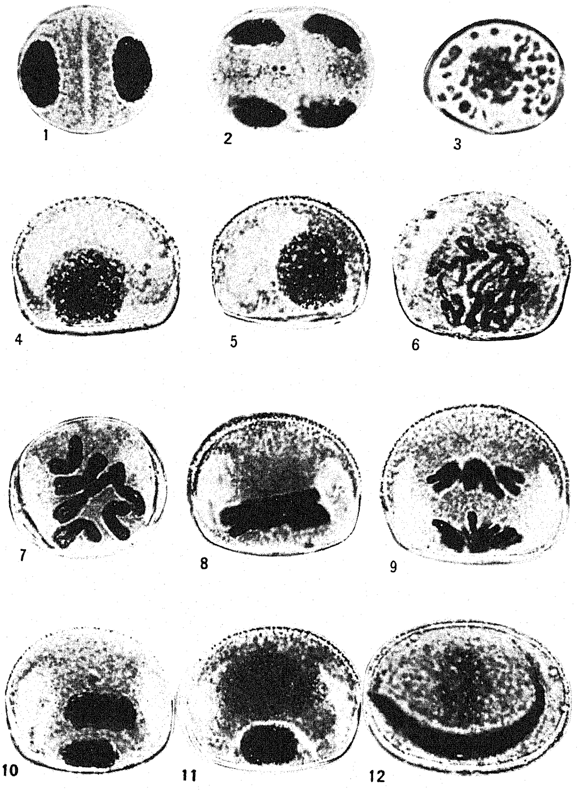
at telophase will be surrounded by "normal" cytoplasm, and this cytoplasm would in no way inhibit the second meiotic division. But the genic material released at the prophase of the second meiotic division would be deficient, so that the microspores would contain "deficient" cytoplasm. All the four microspores resulting from non-disjunction at the first meiotic division would then be deficient for certain essential gene products, and no further development could occur. The theory is plausible, of course, although it is possible that other factors are involved.

Evidence from experimental embryology seems to indicate that there is often a time element required for the reaction between the genes and the cytoplasm (2). This time lag may not be the same in different organisms nor in different stages of development. As the chromosomes become more differentiated, it is probable that each chromosome, or even a small segment of a chromosome, is more essential for the normal development of the individual. The cytoplasmic mass and amount of genic material released at each division may also be factors which determine the time when the cytoplasm responds to gene action.

Summary

1. Development of the male gametophyte in *Tradescantia* seems to be correlated with a polarity established during the meiotic divisions. The spindle of the first microspore division is almost invariably oriented across the short axis of the cell, and the dividing nucleus is nearer the cell wall formed during meiosis. The nucleus formed near this inner cell wall becomes the elongated generative nucleus which divides in the pollen tube to form the two male gametes. The other cell, surrounded by most of the cytoplasm in the microspore, becomes the vegetative or tube nucleus and remains in the resting condition. Development is associated with a movement of the microspore nucleus before division and the formation of characteristic vacuoles.

2. Non-disjunction in segmental interchange species results in chromosome deficiencies in the dyad nuclei, but these nuclei divide normally at the second division, even though they are deficient for a chromosome segment. But the deficient one-nucleate microspores do not develop further. It is suggested that development of the



SAX & EDMONDS on TRADESCANTIA

microspore is dependent on a full complement of gene products which pass into the cytoplasm at the preceding division.

3. A brief chronology of meiosis and microspore development is presented. About two weeks elapse between early meiotic prophase and the formation of mature pollen grains in *Tradescantia reflexa.*

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EXPLANATION OF PLATE IV

FIGS. 1–12.—Photographs of microspores of *Tradescantia gigantea* from aceto-carmine preparations. ×950.

FIG. 1.—Dyad showing position of daughter nuclei at end of first meiotic division.

FIG. 2.—Tetrad stage.

FIG. 3.—One-nucleate microspore showing granules in cytoplasm. Sterile microspores seldom develop beyond this stage.

FIGS. 4, 5.—Microspore showing relations of nuclei and vacuoles.

FIG. 6.—Prophase of microspore division. Nucleus always lies near flattened wall of microspore at this stage.

FIGS. 7, 8.—First microspore divisions.

FIG. 9.—Late anaphase of microspore division showing characteristic position of nuclei.

FIGS. 10, 11.—Differentiation of nuclei. Small nucleus near inner wall invariably produces the generative nucleus. Vegetative or tube nucleus enlarges and passes into resting stage.

FIG. 12.—Mature pollen grain showing elongated generative nucleus and disintegrating vegetative nucleus.

NEW CASES OF APOGAMY IN CERTAIN HOMOSPOROUS LEPTOSPORANGIATE FERNS

W. N. STEIL

(WITH SIX FIGURES)

Apogamy in Pteris flabellata Thunb.

The first case of apogamy in a *Pteris* species was reported by FARLOW (2) in *Pteris cretica* var. *albo-lineata*. The writer (6) reported apogamy in ten *Pteris cretica* varieties and in *P. sulcata* L., *P. quadriaurita* Retz. var. *argyrea* Moore, and *P. parkerii*, in which apogamy was described the following year. This paper now reports the discovery of apogamy in *P. flabellata* Thunb.

DEBARY (1) investigated the spores of a number of *Pteris* species, including those of *P. flabellata*, and reported the sporophyte to be of non-apogamous origin.

The cultural conditions under which most of the prothallia for this investigation were grown, not only of the *Pteris* species but also of the other ferns in which apogamy is reported, have been described elsewhere (7). In some instances the prothallia were grown on the surface of a sterilized nutrient solution in small Erlenmeyer flasks. Since the embryo is always produced apogamously under ordinary cultural conditions, there can be no doubt in regard to the origin of the embryo of *Pteris flabellata*.

The prothallia of this species become typically heart-shaped (fig. 1) and in most respects resemble those of other species of *Pteris* in which apogamy has so far been described. Antheridia are usually produced in large numbers on the smaller prothallia, and occasionally on the larger ones, including those bearing embryos of apogamous origin. The antherozoids are actively motile and apparently normal. Archegonia have not been observed on any of the many prothallia examined.

When the embryo is about to make its appearance, it begins its development on the ventral side of the prothallium and a short distance posterior to the apical notch (fig. 2). Just previous to the first visible evidence of the formation of the embryo, however, an-

other group of small cells appears in the apical notch of the prothallium (fig. 3). During the early stages of development of the embryo, the small group of cells in the apical notch usually produces a projection (figs. 1, 4, 5). This projection frequently develops a tongue-like or cylindrical process, which, when the prothallia are grown in subdued light, may extend forward for a considerable distance. In some instances apogamous embryos were observed to develop on this outgrowth. The embryos thus produced were not always complete, forming a well developed leaf but often lacking a definite root.

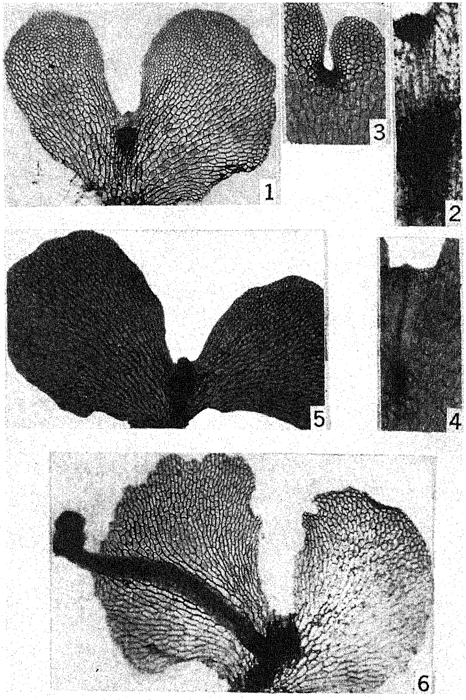
At an early stage in the development of the embryo, tracheids appear in the prothallium, usually a short distance posterior to the apical notch. These vascular elements can be distinguished at first as single isolated cells (fig. 2), but frequently as a compact group of several cells. The tracheids are produced in a considerable portion of the prothallium. Sometimes they extend forward to the apical notch (fig. 4). In a few instances two strands of tracheids were observed to extend toward the apical region of the gametophyte. The presence of these sporophytic tissue elements in a fern prothallium was first reported by LESZCYC-SUMINSKI (1848) in *Pteris sulcata*.

In the order of development of parts of the embryo, the leaf appears first (fig. 5), then the root, and finally the stem. Often a leaf was observed to develop far in advance of the root (fig. 6). No unusual features were found in the later stages of development of the embryo.

The prothallium increases considerably in size during the early stages of development of the embryo, and it continues to enlarge even after the leaf of the embryo has grown upward above the surface of the prothallium.

Apogamy in Pellaea cordata (Cav.) J. Sm.

In 1905 GOEBEL found apogamy in *Pellaea nivea*. Two years later WORONIN (8, 9) discovered apogamy in *P. tenera* and *P. flavens*. In 1910 the writer reported (4) apogamy in *P. atropurpurea* (L.) Link, and later in *P. atropurpurea* var. *cristata* (7), *P. viridis* (Forsk.) Prantl (7), and in *P. adantoidis* J. Sm. (6). PICKETT (3) reported apogamy in *P. glabella*. The gametophyte of *Pellaea cordata*, in



FIGS. 1–6.—*Pteris flabellata:* Fig. 1, prothallium from which an apogamous embryo has been produced; ×20. Fig. 2, dorsal view of portion of prothallium showing apical region and origin of embryo; ×100. Fig. 3, portion of prothallium showing apical region before embryo begins development; ×21.5. Fig. 4, dorsal view of apical region of prothallium showing vascular strand extending to apical notch; ×31. Fig. 5, prothallium showing beginning of leaf of embryo; ×20. Fig. 6, ventral view of prothallium showing that the leaf of the young sporophyte has developed far in advance of root; ×20.

which apogamy was also discovered by the writer, becomes heart-shaped and grows to a large size. The wings of the prothallium have a tendency to become lobed even when light conditions appear to be favorable for the growth of the prothallia.

Antheridia but no archegonia have been observed on the numerous prothallia examined.

From studies so far made, the development of the apogamous embryo of *Pellaea cordata* is similar to that of *Pteris flabellata*.

Apogamy in Cheilanthes feei Moore

So far as the writer is aware, no case of apogamy has heretofore been reported in a *Cheilanthes* species. The gametophyte is considerably smaller than that of either *Pteris flabellata* or *Pellaea cordata*. When the prothallia are grown under the same cultural conditions as those of the other apogamous ferns, they frequently become much branched.

Antheridia are produced in large numbers, but archegonia are never formed.

No unusual features were observed in the development of the apogamous embryo. Occasionally, however, two embryos of apogamous origin were observed to develop from a single gametophyte of *Cheilanthes feei*.

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MÄULE LIGNIN TEST ON PODOCARPUS WOOD

E. C. CROCKER

In 1849, PAYEN (5) noted that when hemp fibers were treated with chlorine water and then with ammonia they became bright red. This is apparently the earliest record of such a reaction, which was advanced as a lignin test by MÄULE (2) in 1901. SCHORGER (7) noted that the wood of angiosperms gave strong red color with the MÄULE test, whereas that of gymnosperms gave only an indefinite brownish gray. The writer (1) confirmed SCHORGER'S observation, and found that the wood of 18 species of angiosperms gave uniformly red coloration, and the wood of 18 species of conifers, and *Ginkgo*, gave indefinite yellow or pale brown color. This distinction has since been further confirmed and extended by SHARMA (8) and SCHINDLER (6), and the MÄULE test has been more or less accepted as a reliable means of distinguishing between the woods of angiosperms and those of gymnosperms.

The writer has recently used the MÄULE test on a great variety of woody fibers from various botanical groups of plants, with interesting results. Many of the rarer of the samples tested were obtained through the kindness of Professor I. W. BAILEY, at the Bussey Institution of Harvard University.

The wood from the many angiosperms tested showed, without exception, strong red color when given the MÄULE test: treatment of shavings with 1 per cent potassium permanganate, followed by washing, treatment with half-strength hydrochloric acid, washing again, and finally treatment with ammonia. The angiosperms tested included the primitive *Trochodendron*, *Drimys*, and *Tetracentron;* also *Monotropa*, and many other herbaceous plants, including members of the Compositae, Labiatae, Ranunculaceae, and Solanaceae. Material was used also from such woody monocotyledons as various bamboos, palms, *Yucca*, and *Ruscus*.

Thorns of members of the Rosaceae, of *Gleditsia*, and *Berberis* failed entirely to give red color, although in the case of *Gleditsia* there was sufficient lignification to give a red color with phloroglucinol reagent. A section of this wood, with a large thorn growing

from it, was given the MÄULE test. The wood colored deep red, but the thorn and its base within the wood showed the yellow-brown negative test only.

It was found that all specimens of the Gnetales gave strong red coloration. Those tested included tracheids from the roots of *Welwitschia mirabilis*, xylem from the stems of *Ephedra gerardiana*, *Gnetum scandens*, *G. vinosum*, and *G. gnemon*. SCHINDLER (6) also secured similar results with the Gnetales.

Xylem from *Lycopodium*, *Aspidium*, *Osmunda*, *Pteris*, *Cycas rumphii*, *C. circinalis*, *Dioon edule*, *Encephalartos hildebrandtii*, *E. villosus*, *E. horridus*, and *Zamia media* gave only the pale brown negative MÄULE reaction, as was also true of the following gymnosperms:

Abies balsamea
Agathis robusta
Araucaria bidwillii
A. cunninghami
A. araucana
Callitris calcarata
Cedrus sp.
Cephalotaxus drupacea
Chamaecyparis thyoides
Cryptomeria japonica
Cupressus macrocarpa
Dacrydium cupressinum
D. franklini
Juniperus virginiana
Keteleeria sp.
Larix laricina
Libocedrus
Phyllocladus hypophyllus
Picea rubens
P. engelmanii
Pinus strobus
P. palustris
P. taeda
Pseudotsuga taxifolia
Sciadopitys verticillata
Sequoia gigantea
Taiwania sp.
Taxodium distichum (?)
Taxus sp.
Thuja occidentalis
Torreya nucifera
Tsuga canadensis

In testing many varieties of coniferous woods, a sample of *Podocarpus pedunculatus*[1] was incidentally encountered which seemed to give a faint reddish coloration, superposed on brown; whereas all other coniferous woods tested up to that time had given only weak brownish color. Numerous repetitions of the test indicated that it was weak but definitely positive.

[1] PILGER (in ENGLER's Pflanzenreich, 1903) gives *amarus* as the accepted species name, and *pedunculatus* as an unaccepted name for the same species. SWAIN (9) used the two names interchangeably.

Through the courtesy of Professor SAMUEL J. RECORD of Yale University, and of Professor H. P. BROWN of Syracuse University, many other samples of *Podocarpus* wood were obtained, all of which (including a specimen of *P. pedunculatus*) gave the usual negative test, excepting two samples of *P. amarus* (from different sources) which gave respectively strong and weak positive tests. Of four samples of this species of wood, from different sources, two gave distinct positive tests, one a weak positive test, and one a completely negative test.

Table I lists the species of *Podocarpus* always giving negative tests.

TABLE I

PODOCARPUS SPECIES GIVING NEGATIVE TESTS

SPECIES NAME (PILGER SYSTEM)*	NAME ON SPECIMENS AS RECEIVED	SPECIMENS EXAMINED
coriaceus	coriaceus	3
dacrydioides	dacrydioides	6
elatus	elata	1
elongatus	elongata	1
ferrugineus	ferruginea	3
gracilior	gracilior	1
guatemalensis	guatemalensis	2
imbricatus	imbricata (2) and cupressina (1)	3
		3
latifolius	latifolia and thunbergii	2
?	lotora	1
macrophyllus	macrophylla	1
montanus	taxifolia	1
nagi	nagi	1
?	nakaii	1
neriifolius	neriifolia (3) and bracteata (2)	5
novae-caledoniae	novae-caledoniae	2
?	philippinensis	2
salignus	chiliana	2
spicatus	spicata	5
totara	totara (8) and hallii (1)	9

* The writer is greatly indebted to Dr. ALFRED REHDER, Curator of the Arnold Arboretum, for checking and comparing species names.

The chemical nature of the material which gives the definite red color in the MÄULE test is still unknown. No estimate has been made of the amount which is present, nor of its possible relation to the material that turns brown in the MÄULE test, which is present in the xylem of conifers, ferns, cycads, and the thorns of *Gleditsia* and

some other angiosperms. It is like phenolphthalein in that its alkaline salt is red-colored and its acid form is colorless.

Some morphologists have surmised that the Gnetales sprang from the Coniferales within or near the *Podocarpus* group. MEZ (3) and his associates, by serological reactions, have come to the same conclusion (4). The findings of the MÄULE lignin test also are consistent with the idea of a possible evolution of the Gnetales from the Coniferales. Further, a particular species (*Podocarpus amarus*) is indicated as the one most nearly like the Gnetales (also the angiosperms, as a group) in exhibiting a particular chemical reaction.

Summary

1. The MÄULE lignin test is apparently a reliable practical test for distinguishing the woods of the conifers from those of the angiosperms and the Gnetales. The only exception found was the single coniferous species *Podocarpus amarus*.

2. Xylem from the three types of Gnetales, *Welwitschia*, *Gnetum*, and *Ephedra*, all give positive MÄULE tests.

℅ ARTHUR D. LITTLE, INC.
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BRIEFER ARTICLES

A NEW METHOD OF DETECTING PATTERNS IN THE RAYS OF FLOWERS

COCKERELL[1] and others have commented on the existence of patterns in the yellow rays of the common sunflower which cannot be detected by ordinary means, the patterns being obscured by the predominance of the yellow pigment. Mrs. COCKERELL discovered a mutant in which this pattern, owing to the unusual development of anthocyanin pigment in the rays, was visible under ordinary conditions. Offspring from this mutant have been cultivated for many years in America, Europe, South Africa, Australia, etc. It was shown by COCKERELL that the pattern in the rays of the ordinary sunflower could be detected in photographs made under certain conditions, even though it could not be seen by direct observation. He suggested that the writer investigate this unusual effect.

It seemed possible that, under certain conditions of illumination, the hidden pattern might be seen directly. In order to test this hypothesis a large Mazda lamp was placed in a projecting lantern mounted in a dark room. A glass water-cell was placed immediately in front of the lantern to remove the infra-red radiation. In front of this was placed at different times many different Wratten Laboratory light filters. These filters, 2 inches square, and made by the Eastman Kodak Company, transmitted only particular wave lengths or particular combinations of wave lengths. If such filters are not available, substitutes can be made by fixing undeveloped photographic plates and staining them with various dyes.

Blossoms of the ordinary sunflower showing no visible pattern in the rays when examined in daylight were examined successively under illumination transmitted by some 60 different filters. When they were examined in the light transmitted by Wratten filter no. 47 the hidden pattern was markedly visible. The rays were green at the tip and red at the base. All of the sunflowers examined showed the pattern although the relative areas of red and green varied with different specimens. Wratten filter no. 47 transmits much of the blue light and a small percentage of the red and green. It transmits practically no yellow, so that the otherwise overwhelming yellow pigment could not obscure patterns which may exist in

[1] Jour. Heredity 6:542. 1915.

other colors. It transmits, however, sufficient of the red and green light to make patterns in these colors discernible. The pattern was not visible in the light from a filter which transmitted only the blue.

The rays of the common sunflowers while illuminated by light transmitted by filter no. 47 were viewed through a glass prism, which separated the images of the red and green colorations. It was then seen that the intensity of the red coloration faded toward the tips of the rays and that of the green increased. Examination of the rays in this manner when illuminated by white light did not show anything satisfactorily because of the overlapping of the colored images, a condition which does not exist to the same degree in selectively filtered light.

This method of detecting patterns may open a fertile field of research and may lead to the development of visible patterns from those which now are hidden and unknown.—JULIAN M. BLAIR, *Department of Physics, University of Colorado, Boulder, Colo.*

CURRENT LITERATURE

Another botanical text-book

Five years of experience with their own classes, and the criticisms, suggestions, and advice from other teachers, have enabled the authors of this widely used text[1] to make considerable change in this edition, while still retaining the general organization and purpose of the book. Some figures have been redrawn and some new ones have been added. The text has been increased, but mostly in the interest of clarity.

It would be interesting in cases like figure 161 to know upon what authority the legend is based. It is doubtless correct, but this stage has been observed so infrequently that botanists would appreciate a reference to the features indicated in the legend. Reconstructions are valuable, pedagogically, but are often inaccurate, as in figures 207 and 222.

The treatment of alternation of generations would seem to give the student a false impression. Plants below the level of sexuality have no alternation of generations. In all plants which have a fusion of gametes, there is an alternation of x and $2x$ phases in the life history. Naturally, at the beginning, as in *Spirogyra*, there is a quick return to the original x condition, and, unless one stops to think, he may not realize that there is any $2x$ phase; but when several cell divisions intervene before return to the x condition, an easily recognizable body with the $2x$ number of chromosomes is built up. The assumption that x and $2x$ genera-

[1] HOLMAN, R. M., and ROBBINS, W. W., Elements of botany. 2d ed. 8vo. pp. viii+404. figs. 268. John Wiley & Sons. New York. 1933.

tions are synonymous with gametophyte and sporophyte generations is responsible for some of the confusion. In such a widely used text, a presentation of the evolution of the $2x$ phase and of the reduction of the x phase in the life history would put more life into the finer morphological part of a book which is so satisfactory in its physiology and in its grosser morphology.

The binding is exceptionally good, since it is not only strong and artistic, but is waterproof and germ-proof.—C. J. CHAMBERLAIN.

Commercial timbers of India

While intended primarily for those interested in the economic utilization of Indian woods, these volumes[2] have a usefulness that will extend to other groups of scientists. The plant geographer will find the distribution maps which are given for each species a most useful and accurate source of information. These maps would provide material for a detailed study of the forest geography of British India.

For each tree species there is given its complete synonymy, including the vernacular names current in various parts of India, and an extensive bibliography which includes literature on the taxonomy, ecology, physiology, and morphology of the species. The wood is described as to its general characteristics, its microscopic structure, its mechanical properties, its working qualities, and its uses, present and prospective. This description is accompanied by photomicrographs. The habit, size, and distribution of each species are given in detail and the distribution is plotted on a base map which includes British India, Burma, and Siam. In this way not less than 320 species are described and illustrated.

An appendix contains a classification of timbers according to use, a glossary of scientific terms and phrases, and a bibliography. Three indexes give access to the material under trade, vernacular, and scientific names, respectively.

Such a collection of information, coming from authors of undoubted qualification, will make the work a classic that will doubtless be the best source of information regarding the forests and forest resources of India for some time to come.

The printing, illustrations, and binding all seem to have been well done, making a volume that will be indispensable to foresters and plant geographers.—G. D. FULLER.

The Gastromycetes in Die Natürlichen Pflanzen-Familien

Volume VIIA of the second edition of Die Natürlichen Pflanzen-Familien[3] has appeared. It is devoted to the Gastromycetes and is a contribution by FISCHER. It is characterized by thoroughgoing workmanship and mature judg-

[2] PEARSON, R. S., and BROWN, H. P., Commercial timbers of India. Vols. I, II. 8vo. pp. 1150. pls. 636. Government of India, Central Publication Branch, Calcutta. 1932. £5.

[3] FISCHER, E. D., Eubasidii: Gastromyceteae. Vol. VII A in Die Natürlichen Pflanzen-Familien (ENGLER) Harms. pp. 122. figs. 91. W. Englemann. Leipzig. 1933.

ment. The volume is printed on good paper; the illustrations are numerous, well chosen, and well done. It is gratifying that this book brings the second edition of the Natürlichen Pflanzen-Familien one step nearer its goal.—G. K. K. LINK.

Systematic botany

It is gratifying to botanists that the last contribution of the late Professor R. WETTSTEIN is being completed by his son. His is a difficult task: to impart essentially the basic ideas of the creator and yet to mould them so as vitally to incorporate the results of new developments in the field of systematic botany. The first volume of the fourth edition of WETTSTEIN's Handbuch der Systematischen Botanik[4] has been made available. The first 159 pages are the last completed contribution of the deceased scholar. The high level of achievement of earlier editions is maintained if not excelled. Fossil plants are considered in detail. The illustrations are plentiful and excellent. The volume is a fine example of botanical book-making.

Part I consists of an interesting and instructive contribution to the basic concepts and propositions of phylogenetic botany. Phylogeny and the evolutionary theory are integrated. In the United States there has been a tendency of late to present these separately, with the result that so-called evolution courses are offered in botanical curricula while phylogeny is languishing.

There is an interesting discussion of the significance of alternation of generations in the phylogenetic development of the plant kingdom. The special part of this volume extends from Schizophyta to the class Gnetinae of the Cormophyta.

It is hoped that time will be found for speedy completion of the concluding volumes of this significant contribution to botanical literature.—G. K. K. LINK.

Tropical woods

Since the previous note[5] regarding this journal, several interesting numbers have appeared.[6] A few of the numerous original articles included in these recent issues are as follows:

STANDLEY, P. C., and STEVENSON, N. S., Two articles on the Cohune palm.

ESPINA, RAMON, and GIACOMETTO, JUAN, Trees of the Sierra Nevada de Santa Marta.

RIMBACH, AUGUST, Forests of Ecuador.

DUCKE, ADOLPHO, Fifteen new forest trees of the Brazilian Amazon.

RECORD, SAMUEL J., Notes on new species of Brazilian woods.

WILLIAMS, L., Peruvian mahogany.

RECORD, SAMUEL J., Notes on tropical timbers.

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[4] WETTSTEIN, R., Handbuch der Systematischen Botanik. pp. iv+537. figs. 555; frontispiece. Franz Deutticke. Leipzig and Vienna. 1933.

[5] BOT. GAZ. 94:429. 1932.

[6] Tropical woods, edited by SAMUEL J. RECORD, School of Forestry, Yale University. 1932, 1933.

STANDLEY, P. C., Three new trees from COLOMBIA.
RECORD, SAMUEL J., The woods of Rhabdodendron and Duckeodendron.
MCLAUGHLIN, R. P., Systematic anatomy of the woods of the Magnoliales.
STANDLEY, P. C., New names for tropical American trees.—A. C. NOÉ.

Life history of flowering plants of central Europe

A previous note[7] reviewed numbers 31 to 36 of a handbook giving the ecological life history of the flowering plants of Germany, Austria, and Switzerland. Since that time eight more serial numbers (Lieferungen) have appeared.[8] They contain the Dioscoreaceae by W. BRENNER, Ulmaceae by H. WALTER, Amaryllidaceae and Iridaceae by F. BUXBAUM, Orchidaceae by H. ZIEGENSPECK, and Moraceae by H. WALTER. The handbook combines in a very successful manner the ecological, morphological, and taxonomic aspects of the plant families, which are treated in the form of rather brief monographs by the individual collaborators. Ample illustrations are supplied.—A. C. NOÉ.

Pleistocene paleobotany

In recent years the study of lake bottoms and swamps by means of pollen analysis has made much progress and has greatly increased our knowledge of glacial and post-glacial plant distribution. A very excellent sample of this kind of investigation[9] combines botanical, geological, and anthropological facts in order to attain a clear conception of the changes in the flora of the region around the Federsee (State of Würtemberg, Germany). Climatic changes and the extinction of former plant inhabitants are discussed, as well as the survival of certain glacial types.—A. C. NOÉ.

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[7] BOT. GAZ. 91:222. 1931.

[8] Lebensgeschichte der Blütenpflanzen Mitteleuropas. Edited by WANGERIN, W., and SCHRÖTER, C., Eugen Ulmer, Stuttgart. 1931–1933.

[9] Paläobotanische Monographie des Federseerieds by KARL BERTSCH. Bibliotheca Botanica, No. 103. E. Schweizerbart'sche Verlagsbuchhandlung, 8vo. pp. viii+126. Stuttgart. 1931. Illustrated.

VOLUME XCV NUMBER 2

THE BOTANICAL GAZETTE

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CYTOLOGY OF THE TRIBE MADINAE, FAMILY COMPOSITAE

DONALD A. JOHANSEN

(WITH ONE HUNDRED AND FOUR FIGURES)

Introduction

The study here reported was begun in the spring of 1931 at the request of Dr. HARVEY MONROE HALL, of the Division of Plant Biology, Carnegie Institution of Washington, who had been occupied for many years previously with a revision of the tribe Madinae of the family Compositae. Dr. HALL had reached the point where he wished to have available information regarding the cytological constitution of certain of the forms whose exact taxonomic disposition had occasioned considerable difficulty. The writer undertook during that year to investigate such forms as were designated by Dr. HALL, plus several additional species which were thought to be deserving of examination. Following Dr. HALL'S unfortunate demise early in 1932, the investigations on the Madinae were continued by his associates, Dr. DAVID D. KECK and Dr. J. CLAUSEN. After the peculiar cytological situation in the tribe was revealed they generously offered the writer the opportunity of investigating all the forms which were available during 1932. They have checked over all matters with which the revision might be concerned. The names for genera, sections, and species have been provided by Dr. KECK.

The present paper is obviously not the proper place for the publication of new species nor for the formal notation of other taxonomic changes. Although the arrangement under each of the various genera

will reveal to a certain extent the eventual disposition of its individual species, what has thus been indicated should not be understood to be final, for much work yet remains to be performed before such action becomes possible. In order that the final monograph and what is presented in this paper may be co-ordinated, the field numbers and localities of most of the forms studied have been cited; the few exceptions are in the cases of certain species, such as *Hemizonia angustifolia*, in which such a large number of plants were examined that space cannot be spared for their citation.

Materials

By far the greater proportion of the plants upon which this study is based were originally collected by Dr. HALL and grown in the garden of the Carnegie Institution on the Stanford University campus. All numbers in the 12th and 13th thousands cited herein are Dr. HALL'S collection numbers. The species secured by Dr. KECK bear his own collection numbers preceded by his surname (for example, *Keck 1323*). Those collected by Mr. WM. M. HEUSI of the Institution staff, who was in charge of the cultivation of all the garden plants, are similarly denoted (for example, *Heusi 110*). Vouchers of all plants examined cytologically have been preserved. The writer's own collections of material were made directly from the plants in the field. At the time of obtaining the material, vouchers were also made and given the writer's collection numbers (for example, *DAJ 690*). All such specimens were examined and identified by Dr. KECK.

It has been impossible to obtain either seeds or suitable cytological material of the two Hawaiian representatives of the Madinae, *Argyroxiphium* DC. and *Wilkesia* Gray. Representatives of all the Californian and Mexican genera with the exception of the monotypic genus *Holocarpha* Greene have been available. *Blepharipappus scaber* Hooker, placed in the Madinae by JEPSON (3), is not considered to belong in the tribe, and has not been examined cytologically.

Except as otherwise indicated, all the localities cited are within the state of California. As a rule the county is not given save when confusion might result, or when there are two distinct localities bearing the same name.

In this paper the genera have been arranged alphabetically, and the sections and species are similarly disposed under each genus. No attempt has been made at this time to evolve a phylogenetic scheme for the tribe.

Methods

In the earlier phases of the work, root tips were obtained by placing young seedlings in Knop's solution; but in the later stages, because of the large number of plants to be examined, the individual plants were simply knocked out of the pots in which they were growing and root tips obtained from the mass of roots to be found between the soil and the pot. The water-culture tips were more favorable from the technical standpoint, in that the chromosome plates were more numerous and the elements more evenly spaced.

In 1931, Taylor's modified chrom-acetic plus 1% of either maltose or urea was principally used as killing fluid for the tips, but in 1932, Nawaschin's fluid was substituted throughout. It was soon found that none of the staining methods in common use was satisfactory, consequently it became necessary to devise one more reliable (4). As finally worked out, the method has been improved to the extent of substituting methyl violet 2B for "gentian violet" so-called, and using erythrosin B both as counterstain and to differentiate the violet a little further (both stains were from Coleman and Bell). A clear, sharp stain of optimum intensity invariably resulted. The buds were removed from the plant and the upper portion of the capitulum sliced off. They were then dropped into Carnoy's fluid, whence, after about 8 minutes, they were transferred to Nawaschin's fluid. The fixation was ordinarily excellent, but Nawaschin's fluid occasionally proved erratic in its action. The buds were stained exclusively with methyl violet 2B alone. When possible, and in general, both haploid and diploid counts were made on the same plant; but the drawings of the somatic and meiotic chromosomal complements in any particular form are not necessarily from the same plant.

The preparations were examined and the drawings prepared in the cytological laboratory of the Carnegie Institution, using a Zeiss binocular microscope equipped with a Leitz achromatic-aplanatic condenser, aperture 1.4; Zeiss objective ×120, aperture 1.3, and

Mobimi oculars ×20, giving a bench-level magnification of approximately 4400. "Parallux" parallel illumination was employed.

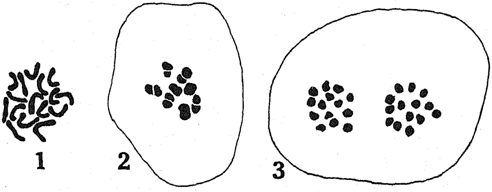
Previous work on the Madinae

There exists only one report of earlier work on the cytology of the Madinae, that of MANN (BABCOCK and HALL 1). This was at best not much more than an exploratory examination, and the preparations employed were of a temporary character. MANN reported finding 12n chromosomes in each of three subspecies of *Hemizonia congesta*, namely, *typica*, *lutescens*, and *luzulaefolia* and 10n chromosomes in *Hemizonia corymbosa* (=*H. angustifolia*). As will be noted later, the counts for *H. angustifolia* have been amply confirmed, but those for the subspecies of *H. congesta* are apparently erroneous.

Observations

ACHYRACHAENA Schauer

This monotypic genus only superficially resembles the other Madinae. The species, *A. mollis* Schauer, an annual, proved very



FIGS. 1–3.—*Achyrachaena mollis:* fig. 1, SM* (2n-16); fig. 2, MI (n-8). *Hemizonia*, section Blepharizonia. *H. plumosa:* fig. 3, MII (n-14). ×2200.

* The following abbreviations are employed in the descriptions of figures: SM, somatic metaphase; MI, first meiotic metaphase; MII, second meiotic metaphase; AI, first meiotic anaphase.

difficult to study cytologically. The root tips are poorly constructed and satisfactory figures are rare; it is also hard to find clear, well stained plates at either the first or the second metaphase of the meiotic divisions. The material principally studied was *13058*, from Priest Valley, supplemented by examination of material collected by the writer on the Stanford campus and on Mt. Hamilton. The haploid number is 8, the diploid 16 (figs. 1, 2). The somatic chromo-

somes are small, curved, and so crowded together that few do not overlap. For this reason it is difficult to make exact computations and the number occasionally appeared to be 17 or 18 rather than 16. Trabants ordinarily could not be seen.

HEMIZONELLA Gray

The genus consists of but one species, *H. minima* Gray, a low, montane annual. Only a single plant, which originally came from Mather, was available. Both the root tips and the few buds secured were very unsatisfactory, and it was not possible to make accurate counts in either. Several metaphase plates showed approximately 26 somatic chromosomes. In the microsporocytes, no first or second metaphase plates were encountered. In each of three interphases, however, 13 chromatic bodies were definitely countable.

HEMIZONIA DeCandolle

The genus is readily separable into six sections which are fairly consistent systematically but not always so cytologically. The sections include: Blepharizonia, Calycadenia, Centromadia, Euhemizonia, Fruticosi, and Hartmannia (in part). All the sections contain only annual plants, with the exception of the Fruticosi, which are perennials.

Section BLEPHARIZONIA Gray

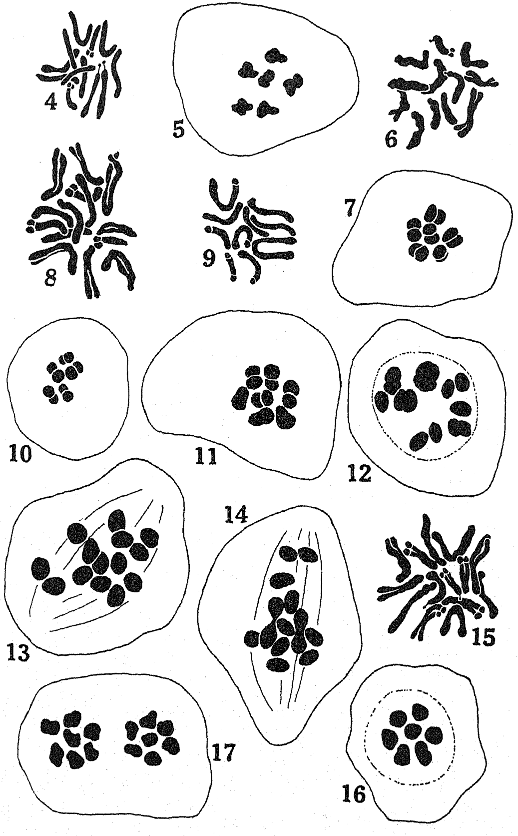
The section contains only the following:

H. plumosa (Kell.) Gray. n-14.—A single garden plant provided buds in which the haploid number was best counted at the second metaphase (fig. 3).

Section CALYCADENIA Gray

The section consists essentially of the genus *Calycadenia* of DeCandolle and contains perhaps twelve species, of which six have been subjected to cytological examination.

H. bicolor Jepson. n-6, 2n-12.—Four collections were studied; they proved to be identical in all respects cytologically. The somatic chromosomes are noteworthy for their length as compared with their breadth, among the Madinae. Two chromosomes appear to be medianly and two submedianly constricted, but the fixation (chromacetic) was apparently not good enough to bring out all the morpho-



FIGS. 4–17.—*Hemizonia* section Calycadenia. *H. bicolor:* fig. 4, SM (2n-12); fig. 5, MI (n-6). *H. ciliosa:* fig. 6, SM (2n-12); fig. 7, MI (n-6). *H. mollis:* fig. 8, SM (2n-14). *H. pauciflora:* fig. 9, SM (2n-10); fig. 10, MI (n-5). *H. truncata:* fig. 11, MI, normal with 7 bivalents; fig. 12, late diaphase with 4 bivalents and 6 univalents; fig. 13, AI with 14 univalents; fig. 14, AI with 2 bivalents and 10 univalents. *H. truncata* ssp. *"scabrella":* fig. 15, SM (2n-14); fig. 16, late diaphase (n-7). *H. villosa:* fig. 17, MII (n-7). ×2200.

logical characteristics (fig. 4). Two trabants are always prominently to be seen. The meiotic chromosomes are somewhat irregular at the first metaphase (fig. 5); at an early stage in the second metaphase there is evidence of secondary association.

H. ciliosa Jepson. n-6, 2n-12.—Four groups of plants were available for examination. As in *H. bicolor*, there is one pair with median and another with submedian constrictions, while the remaining chromosomes appear to lack such characters. The trabants are very tiny and on a short, stout, terminally constricted portion of the chromosome (fig. 6), whereas those in *H. bicolor* are on long threads. Meiotic behavior is regular (fig. 7).

H. mollis Gray. n-7, 2n-14.—Four sets of plants were examined, but all proved to be cytologically identical. The somatic chromosomes of this species are large, and all save possibly one pair have definite submedian constrictions (fig. 8). In most of the chromosomes the longitudinal split appears in late prophase. Trabants are apparently not of regular occurrence.

H. pauciflora Gray. n-5, 2n-10.—This species was studied during 1931, only one plant being available, *12936*, which came originally from near Stonyford, Colusa County. Fixation of the somatic chromosomes was not perfect enough to reveal minute details of the morphology, but it is nevertheless clear that the chromosomes may be grouped according to length into the following pairs: one long U-shaped, one medium long, two short, and one still shorter (fig. 9). These differences are perpetuated in the sizes of the meiotic chromosomes (fig. 10), as the latter include the following sizes: one large, one medium large, two small, and one slightly smaller.

H. truncata Gray. n-7, 2n-14.—Four groups of plants were examined: *12860* from north of Plymouth, Amador County, *12862* from west of Placerville, *Heusi 107* from north of Yountville, and *13238* from hills back of Post's, Monterey County. The somatic divisions were difficult to study; there were practically no clear, flat metaphase plates to be found in the only group of which root tips were studied (*13238*). The diploid number, at any rate, is certainly 14. In one or two instances a chromosome was observed to have been split transversely into two equal portions, but there is no real evidence that fragmentation occurs in this species.

In *12860* (fig. 11) and *12862* meiosis seemed to be perfectly regular, but it was markedly abnormal in *Heusi 107*. In some microsporocytes all the chromosomes existed as univalents, while the following assortments of bivalents and univalents were observed in other mother cells: 2_{II}-10_{I}, 3_{II}-8_{I}, 4_{II}-6_{I}, and 5_{II}-4_{I}. It will be noticed that in each case the total number comes to seven pairs. Some of these configurations are illustrated (figs. 12-14).

H. truncata ssp. "*scabrella*." This subspecies is the equivalent of *H. scabrella* Drew. n-7, 2n-14.—The somatic chromosomes are clearer than in the species, but it is still difficult to diagnose their morphology. Two are invariably bent sharply at the center, but it is not known whether constrictions actually occur there. Others are submedianly to subterminally constricted. The two terminally constricted chromosomes carrying the very small trabants are distinctly the shortest in the entire complement (fig. 15). The meiotic chromosomes seem to be perfectly regular in their entire behavior (fig. 16).

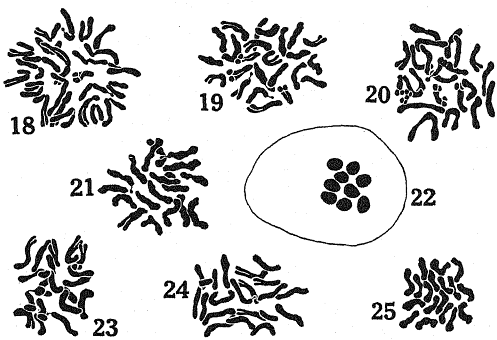
H. villosa Jepson. n-7, 2n-14.—The one group of plants examined, *13157*, was originally secured in Jolon Valley. The somatic chromosomes are rather large, but too interlaced for illustrative purposes. Two terminally constricted chromosomes carry prominent trabants. The meiotic chromosomes at the first metaphase lie too close together, but those at the second metaphase are clearly separated (fig. 17). No morphological differences were observable at either stage or in the somatic mitoses.

Section "CENTROMADIA" (Greene)

The section is equivalent to the genus *Centromadia*, as proposed by GREENE, and includes a homogeneous group of very spinescent species. As most commonly recognized up to the present time, the former genus contained two species, *C. fitchii* (Gray) Greene and *C. pungens* (T. & G.) Greene, the latter including two varieties. *C. fitchii* has unfortunately been unavailable, but a number of forms of *C. pungens* have been examined. From the cytological standpoint, the reduction of *C. maritima* Greene to synonymy under *C. pungens* appears to be justified, although in the present paper it is accorded separate treatment. However, neither of the two varieties, *congdoni*

and *parryi* (3), can be retained as such, but are being restored to their original rank as distinct species, because of the unexpected discrepancy in chromosome numbers.

H. congdoni Rob. & Greenm. 2n–24.—The four groups of plants studied and the localities at which they were originally collected were: *13190* from 4 miles southwest of Salinas, *13193* from south of Salinas, *13273* at Chualar, and *13274* from south of Castroville. These localities are all within the lower part of the Salinas Valley,



FIGS. 18–25.—*Hemizonia* section Centromadia. *H. congdoni:* fig. 18, SM (2n-24) from *13273*, and fig. 19, from *13190*. *H. parryi:* fig. 20, SM (2n-24). *H. pungens:* fig. 21, SM (2n-24); fig. 22, MI (n-9). *H. pungens* ssp. "*maritima*": fig. 23, SM (2n-19 ? with 3 pieces) and fig. 24, SM (2n-21), both from *13261;* fig. 25, SM (2n-18). ×2200.

and incidentally within the range of *H. pungens.* In the somatic complements, *H. congdoni* possesses two long submedianly constricted chromosomes, exactly as in *H. parryi;* in the first species they are always in the center of the plate, but on the periphery in the latter. In *13190* this peculiar pair was not definitely recognizable in most of the plates examined (fig. 19). The plates of *13273* (fig. 18) were the most satisfactory, and the following characterization is based upon them: 2 very long with submedian constrictions, 8 with median, 12 with submedian, and 2 with subterminal. Two of the medianly constricted chromosomes in addition possess terminal con-

strictions ending in fairly large trabants. In some of the medianly or submedianly constricted chromosomes, the constricted part may become drawn so fine that the two portions separate. This provides an explanation for the occasional counting of 25 or 26 chromosomes.

H. parryi Greene. 2n–24.—The plants studied, *13277* and *Keck 1291*, were originally secured west of Alviso and at Salada Beach respectively. The somatic complements are noteworthy for the presence of two very long chromosomes which are medianly constricted and which apparently often fragment at this juncture, so that in many plates 26 chromosomes were counted (fig. 20; the long pair is at the lower left). Ordinarily only a single large trabant is to be observed.

H. pungens T. & G. n–9, 2n–18, with irregularities at meiosis.—Eleven different collections were examined: *12847*, *12907*, *12959*, *13192*, *13260*, *13264*, *13278*, *13279*, *J. J. Walters* at Bard and at Little Shasta River, and *DAJ 689*. The somatic chromosomes are short, thickish, more or less sharply bent or curved, and most of them apparently possess more than one submedian constriction (fig. 21). This character is more pronounced in some of the plants than in others; only a laborious statistical survey of innumerable plates could provide a basis for the morphological classification of the chromosomes. Two terminally long-constricted chromosomes carry large trabants. In two clear cases only 17 chromosomes could be counted. Meiosis has revealed many irregularities: cytomixis is common in the collection from Bard; irregularities in the distribution of the chromosomes to the poles at the first division are common; bivalents are irregularly oriented on the spindle; and often a small blob of chromatin is found in the cytoplasm at the first anaphase or telophase. The figure (fig. 22) is from a plant in which behavior was regular and normal. Rarely two to four univalents may be seen. In one plant, only 8n chromosomes were counted in many of the microsporocytes; this was doubtless due to simple fusion, since one chromosome was greatly swollen. At late metaphase or early anaphase of the first division in *12847*, it was interesting to observe that in most of the cells there were four bivalents with one chiasma and five with two chiasmata; less frequently there were three with two chiasmata and six with but one chiasma.

H. pungens: the "*maritima*" form (=*Centromadia maritima* Greene). n–9, 2n–18, with evidence of fragmentation.—Three series of plants were examined, all coming from the region on both sides of the southern arm of San Francisco Bay: *13259*, *13261*, *13262*. In the somatic complements of *13262*, no evidence of trabants was found (fig. 25), while the plants of the other two groups all showed a single chromosome with one such appendage (figs. 23, 24). In the latter plants, moreover, two or three chromosomes are subterminally shallowly constricted at one end and similarly long-constricted at the other end (fig. 23), separating off a large subglobular piece. This portion may become detached, whereupon it apparently changes its shape to resemble a short chromosome (fig. 24). As there is a total of 21 chromosomes in many plates, some other chromosome has apparently (probably medianly) fragmented (fig. 24).

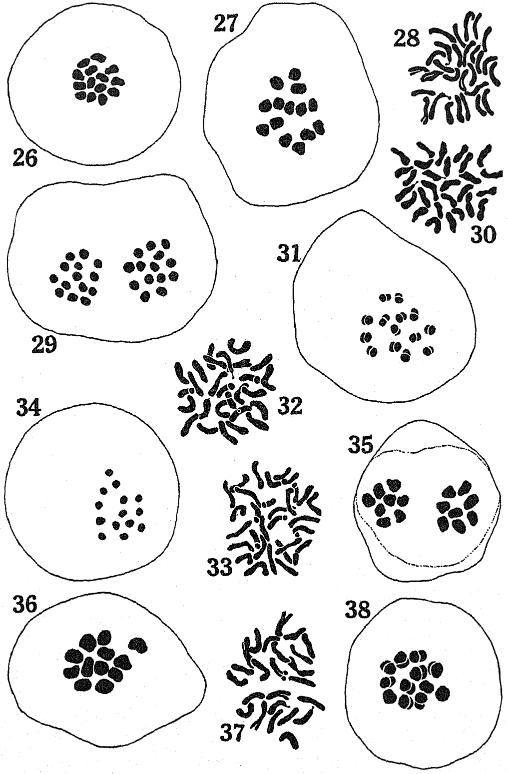
Section Euhemizonia Gray

The section contains two species which differ greatly in their chromosome numbers, but the number has not been found to vary within the individual species.

H. congesta DC.—The species is divided into six subspecies, of which all except ssp. *tracyi* Babcock & Hall have been examined. As indicated in a preliminary paragraph, no plants have been found in any subspecies of *H. congesta* with 12n chromosomes, as reported by Mann (1). All the subspecies have consistently shown 14n chromosomes in every plant examined.

H. congesta ssp. *calyculata* Babcock & Hall. n–14, 2n–28.—Several plants of *12945*, found on the Orr's Spring Road, Mendocino County, provided the material. The somatic chromosomes are the morphological duplicates of those of ssp. *lutescens*. A few have submedian or subterminal constrictions. The two trabants are very small and on long threads. The meiotic chromosomes are intermediate in size between those of ssp. *lutescens* or *clevelandi* and *luzulaefolia*, and of all the subspecies are placed closest together at the first metaphase (fig. 26).

H. congesta ssp. *clevelandi* (Greene) Babcock & Hall. n–14.—Buds of *12926* were examined, from the Eel River east of Laytonville. The meiotic chromosomes are well isolated and easily countable (fig. 27); they are of a size with those of ssp. *lutescens*.



FIGS. 26–38.—Figs. 26–35, *Hemizonia* section Euhemizonia. *H. congesta* ssp. *calyculata:* fig. 26, MI (n-14). *H. congesta* ssp. *clevelandi:* fig. 27, MI (n-14). *H. congesta* ssp. *lutescens:* fig. 28, SM (2n-28); fig. 29, MII (n-14). *H. congesta* ssp. *luzulaefolia:* fig. 30, SM (2n-28); fig. 31, MI (2n-14). *H. congesta* ssp. *typica:* figs. 32, 33 from *13079*, showing respectively fusion (2n-27) and fragmentation (2n-30); fig. 34, MI (n-14). *H. wheeleri:* fig. 35, MII (n-8). Figs. 36–38, *Hemizonia* section "Fruticosi." *H. clementina:* fig. 36, MI (n-12). *H. greeneana:* fig. 37, SM (2n-24); fig. 38, MI (n-12). ×2200.

H. congesta ssp. *lutescens* (Greene) Babcock & Hall. n–14, 2n–28. —The plants which provided the material, *13104*, came from Bodega Bay. As with ssp. *typica*, it is impossible to determine much of the intimate morphology of the somatic chromosomes (fig. 28), although there is considerable evidence of submedian constrictions in most of the chromosomes and the attachment fibers appear to be located at these points. No irregularities in mitosis were noted. Two medium sized trabants occur. There were no clear polar views of the first meiotic metaphase in the available material, consequently a similar aspect of the second metaphase is being presented (fig. 29).

H. congesta ssp. *luzulaefolia* (DC.) Babcock & Hall. n–14, 2n–28. —Four groups of specimens provided abundant material. In most of the somatic complements in all of the plants the chromosomes were too close together and too much intertwined for wholly accurate counts. As the chromosomes themselves are rather small, it was difficult to obtain positive evidence of the occurrence of fragmentation when the total number appeared to exceed 28. The trabants are exceedingly tiny, but could always be located (fig. 30). The meiotic chromosomes are spaced far apart (fig. 31), and are a little larger than those of ssp. *typica*.

H. congesta ssp. *typica* Babcock & Hall. n–14, 2n–28, with apparent fusion and fragmentation.—The three plants studied were: *13076* from the Gallinas Valley, Marin County, *13079* northwest of Petaluma, and *13263* on the Bodega Road, Sonoma County. It is very difficult to judge the true number of chromosomes in most of the somatic complements, principally because of overlapping and intertwisting. In all the root tips examined, the range in number was from 24 to 30. In the clearest plates, 28 were indubitably counted; when the number drops to not more than 27 (fig. 32), one suspects the occurrence of fusion, and when it exceeds 28 (fig. 33), the presence of shorter elements indicates that fragmentation took place. Although at anaphase practically all the attachment constrictions are submedian, there is little evidence of constrictions at metaphase. Two large trabants are present. The very small meiotic chromosomes are always widely separated at the first metaphase (fig. 34), so that exact counts are readily made; no variations in the number have been noted.

H. wheeleri Gray. n–8.—The buds were secured from garden plants late in the season; it was impossible to obtain satisfactory root tips. The plants studied, *13820*, were originally collected at Mineral King. The meiotic chromosomes are large but more easily countable at the second metaphase (fig. 35).

Section "FRUTICOSI"

The section consists of six perennial species, mostly insular, of which those examined possess the same chromosome numbers and seem to be very close together cytologically.

H. clementina Brandegee. n–12, 2n–24.—The two sets of plants available, *Heusi 110* and *111*, were originally obtained on Santa Catalina Island. Estimations of the somatic number were difficult to make; each of the 24 chromosomes has a deep and rarely long submedian constriction, at which point the shorter arm is bent around and often twisted, and as a rule the elements are badly crowded together. The meiotic chromosomes are comparatively large but of the same size throughout (fig. 36).

H. greeneana Rose. n–12, 2n–24.—The plants were grown from seed secured by J. T. HOWELL on Guadalupe Island, Mexico (*S315*). The somatic chromosomes are too short and thick for the facile identifications of constrictions (fig. 37), but at least two pairs plainly show submedian ones. The two chromosomes bearing the terminal trabants are easily located. Meiosis shows some irregularity; the bivalents separate early in the first metaphase (fig. 38).

Section HARTMANNIA Gray, *pars*

The section is the largest in the genus, but it is problematical how many species it contains because of the extreme difficulty of establishing demarcatory lines between certain of the species, notably *H. virgata* and *H. heermanni*, on both taxonomical and cytological grounds. The section is unique for the wide variations in chromosome numbers to be found amongst its various species.

H. angustifolia DC. n–10, 2n–20.—About 22 distinct groups of plants were examined; all proved to be cytologically homogeneous. There is considerable variation in the bulk and size of the somatic chromosomes in the different plants, as might be expected. They may be either short and plump or long and narrow, more or less

curved, with at least two pairs definitely shorter than the remainder, but with no certain evidence of constrictions or trabants (figs. 39, 40). The meiotic chromosomes are large, two of them seeming to be a trifle less bulky than the others (fig. 41).

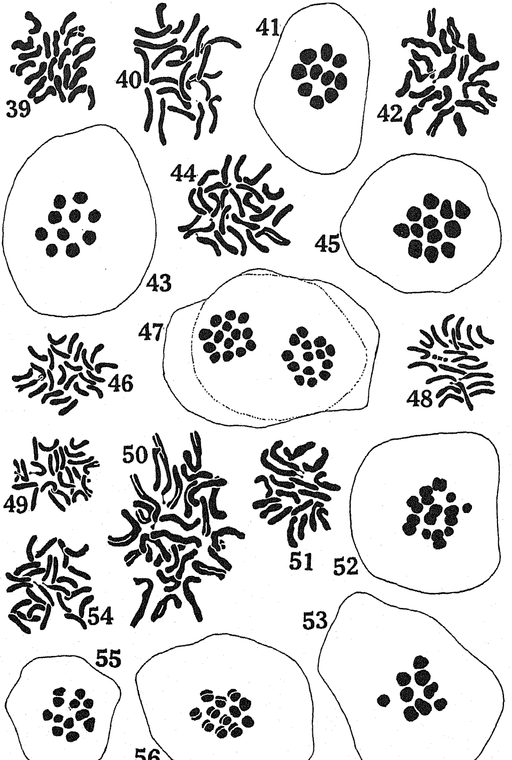
H. angustifolia: the "*macrocephala*" form (=*H. macrocephala* Nutt.). n–10, 2n–20.—This subspecies occurs along the coast in northwestern San Luis Obispo County, the three groups of plants examined coming from San Simeon (*13150*) on the north, through Cambria (*13153*), southerly to Morro (*13140*). Both the somatic (fig. 42) and the meiotic (fig. 43) chromosomes are similar to those of the typical species, but in the somatic set *macrocephala* always possesses a pair with terminal constrictions ending in small trabants. Size differences appear to be somewhat more pronounced in the subspecies. Meiosis is perfectly regular.

H. fasciculata (DC.) T. & G.—This species is composed of a number of forms which were provisionally separated by HALL as distinct subspecies. It soon became evident, however, that the subspecies did not all contain the same chromosome numbers. It is clearly apparent that far more detailed studies upon a large number of individuals are necessary before any taxonomic conclusions with a cytological basis are possible.

H. fasciculata ssp. "*ramosissima.*" This is equivalent to the variety *ramosissima* (Benth.) Gray. n–12, 2n–24.—Eight groups of plants were examined and found to be consistent cytologically. The somatic chromosomes are rather long with perfectly smooth contours. In all the plants save one, two elements were abruptly and terminally short-constricted to bear small trabants (fig. 44). The meiotic chromosomes at the first metaphase are regularly organized (fig. 45).

H. fasciculata ssp. "*superfasciculata.*" 2n–24.—Only the root tips of one group of plants, *13019*, from Ramona, were examined in 1931. The somatic chromosomes (fig. 46) are similar to those of ssp. *ramosissima* but smaller, and usually only one chromosome bears a trabant.

H. fasciculata ssp. "*lobbi.*"—This subspecies is the var. *lobbi* (Greene) Gray. Three groups of plants were examined: *12844* from Livermore Valley, *13043* from Cook P.O., San Benito County, and



FIGS. 39–56.—*Hemizonia* section Hartmannia (*pars*). *H. angustifolia:* fig. 39 (chromacetic fixation) and fig. 40 (Nawaschin's), SM (2n-20); fig. 41, MI (n-10). *H. angustifolia* ssp. "*macrocephala*": fig. 42, SM (2n-20); fig. 43, MI (n-10). *H. fasciculata* ssp. "*ramosissima*": fig. 44, SM (2n-24); fig. 45, MI (n-12). *H. fasciculata* ssp. "*superfasciculata*": fig. 46, SM (2n-24). *H. fasciculata* ssp. "*lobbi*": fig. 47, MII (n-14); fig. 48, SM (2n-22). *H. fasciculata* ssp. "*hispidula*": fig. 49, SM (2n-22). *H. floribunda:* fig. 50, SM (2n-26). *H. kelloggii:* fig. 51, SM (2n-18); fig. 52, MI showing 7 bivalents and 4 univalents. *H.* "*pallida*": fig. 53, MI (n-9). *H. paniculata:* fig. 54, SM (2n-24); figs. 55, 56, MI (n-12) (fig. 55 is from *13041* and fig. 56 from *13134*). ×2200.

DAJ 691 from 6 miles north of San Benito. In the last collection meiosis was found to be extremely irregular, and as it was thought to have been influenced by the high temperatures prevailing at the time of collecting and that fixation might have been rendered inadequate by the excessive stickiness of the buds, this lot of material was discarded. In *12844*, buds only were available in 1931 and showed the meiotic chromosomes to number 14; the second metaphase plates were more suitable for countings (fig. 47). In *13043*, the root tips all possessed 22 diploid chromosomes (fig. 48), although on two plates not more than 20 could be counted. In most of the plates two pairs are submedianly constricted and two small trabants are present. The subspecies appears to be a good one, yet there seems no way at present of explaining the two widely different sets of chromosome numbers, 11n and 14n.

H. fasciculata ssp. "*hispidula.*" 2n–22.—In this group only root tip counts are being reported, because of the unsuitability of nearly all of the bud material preserved. Three groups of plants were examined: *13205* (4 miles north of Bradley), *13042* (Atascadero Hills), and *13206* (2.2 miles west of Atascadero). In the first two collections, the 22 chromosomes were identical in size, while those in *13206* were much larger, being nearly as large as those in the 24-chromosome sets in other subspecies. In all these 22-chromosome plants only one morphological peculiarity was noticed, namely, that the two chromosomes with trabants have a submedian constriction, at which point the shorter arm is bent around sharply and is terminally long-constricted into the medium sized trabants (fig. 49). In *13206*, two chromosomes possess shallow constrictions but no trabants. It would seem that the behavior of the trabants is deserving of particular attention in future studies on *H. fasciculata*.

H. floribunda Gray. n–13, 2n–26.—The only plants available, *13010*, were originally secured at Potrero, San Diego County. Most if not all of the somatic chromosomes (fig. 50) appear to have submedian constrictions; two are terminally long-constricted to carry fairly large trabants. About four pairs are markedly shorter than the other chromosomes. A considerable number of buds were preserved but all save one had too young flowers; in the one exception the fixation was unsatisfactory. In the few first metaphase plates in

which an approximate estimation was possible, 13n chromosomes were to be seen.

H. kelloggii Greene. n–9, 2n–18.—The one group of plants examined, *13025*, was secured at Aguanga Hills, Riverside County. The root tips were not in the best condition but the accuracy of the count cited is unquestionable (fig. 51). In all of the clear first metaphase figures, the chromosomes were disposed as seven bivalents and four univalents (fig. 52). At the first anaphase, two of the univalents appear to go to each pole undivided.

H. "pallida." n–9.—This undescribed species is closely related to *H. angustifolia* and was for a long time regarded as merely a vernal form of the latter; it is of limited occurrence and distribution. The two species, however, are readily separable on both morphological and cytological grounds. The material was collected from plants growing along the roadside 5 miles north of Grapevine, Kern County (*DAJ 676*). First metaphase stages are exceedingly rare, although diaphases and anaphases are found in abundance. In the one illustrated (fig. 53) there is a distinct gradation in the size of the chromosomes, and the same thing was noticed in the first anaphase chromosomes.

H. paniculata Gray. n–12, 2n–24.—Twenty-four sets of plants from various widely separated localities were investigated; all of these except one agreed among themselves in cytological details. One form, *13134*, from 2.7 miles north of Lompoc, showed very distinct evidence of fragmentation existing as a usual occurrence as practically all the somatic plates contained between 26 and 28 chromosomes. The meiotic chromosomes of this form (fig. 56), however, totaled 12 and showed no evidence of any irregularities.

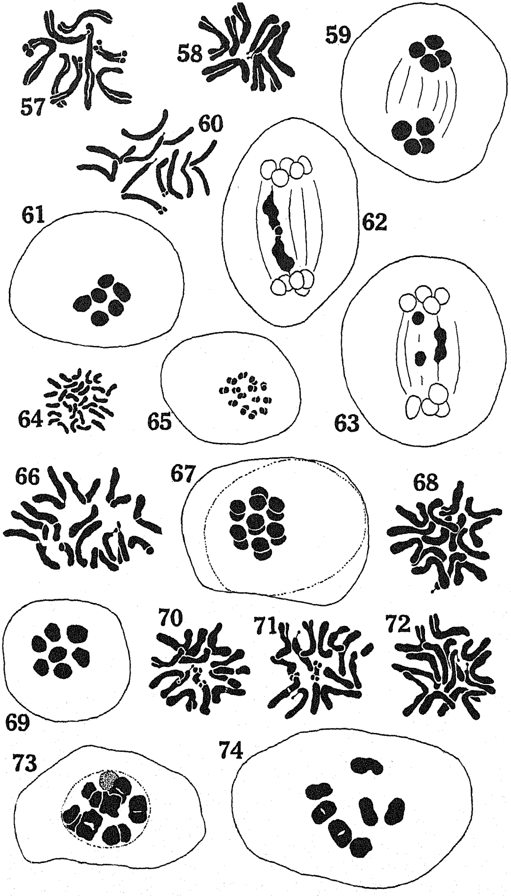
In the majority of the plants the chromosomes displayed no characteristic morphological structure (fig. 54), but in *13284*, from 1.5 miles north of Temecula, four of the chromosomes each showed a deep submedian constriction. This material appeared to be exceptionally well fixed. In *13125*, from Santa Maria, many of the metaphase plates seemed to have one more or one less than the normal number. In all the plants the two terminally constricted chromosomes, which are almost invariably at or near the center of the plate, bearing the small trabants, are easily recognized.

No irregularities of any sort were noticed during meiosis (fig. 55), but in certain more robust plants the chromosomes are twice the bulk of those in less sturdy individuals.

H. paniculata ssp. "*increscens*." 2n–24.—The only recognizable difference between this undescribed subspecies (*13244*, from 4.4 miles north of Salinas) and the species is that the termini of the somatic chromosomes are short-attenuate rather than obtuse. Other morphological details are as described for the species.

H. virgata Gray and *H. heermanni* Greene.—Exactly how to distinguish between these two species is at the moment an unsolved problem. Dr. HALL, upon more than a few occasions, voiced his great perplexity concerning them; he was strongly of the opinion that they belonged together yet could not bring himself definitely to unite them, and was hopeful that the cytological investigation of each might provide a way out of the impasse. Four forms of *virgata* each had 4 haploid and 8 diploid chromosomes, while five of *heermanni* showed 6 haploid and 12 diploid chromosomes; hence it appeared that the two species could be retained as distinct on the basis of that difference in chromosome numbers. This presumption, however, was revealed as fallacious when early in 1933 an indubitable form of *virgata* was discovered by J. CLAUSEN to possess the *heermanni* chromosomal complement. Perhaps the best temporary solution of the problem is to assume that *virgata* and *heermanni* together constitute a single species possessing two distinct cytological forms, plus a third form which has the taxonomical characters of one species but the chromosomal number of the other.

The chromosomes of *H. virgata* may be identified as one very long pair with median constrictions, two pairs with submedian constrictions, plus another pair carrying trabants but which do not appear to be constricted elsewhere than at the terminus (figs. 57, 58, which are from different plants). In *H. heermanni* the somatic chromosomes are narrower, the trabant-bearing pairs are similar to those in *virgata*, and there are likewise two pairs with distinct submedian constrictions, while the remaining three mostly short pairs show no evidence of constrictions (fig. 60). Except for the smaller size of those in *heermanni*, the meiotic chromosomes are similar in the two forms (figs. 59, 61).



FIGS. 57–74.—Figs. 57–65, *Hemizonia* section Hartmannia, continued. *H. virgata:* fig. 57, SM (2n-8), from *13014;* fig. 58, SM (2n-8), from *12885;* fig. 59, late AI (n-4). *H. heermanni:* fig. 60, SM (2n-12) (chrom-acetic fixation); fig. 61, normal MI (n-6); figs. 62, 63, from *12968*, showing irregularities in first division. *Holozonia filipes:* fig. 64, SM (2n-28); fig. 65, MI (n-14). Figs. 66–74, *Lagophylla*. *L. dichotoma:* fig. 66, SM (2n-14); fig. 67, MI (n-7). *L. glandulosa:* fig. 68, SM (2n-14); fig. 69, MI (n-7). *L. ramosissima:* figs. 70, 71 (from *13093*), showing fragmentation of somatic chromosomes; figs. 73, 74: diaphase and transition from diaphase to MI

In *12968*, a true *heermanni*, there are marked irregularities during meiosis. Many mother cells do not undergo meiosis. Lagging chromosomes, delayed separation of bivalents, and similar phenomena are not uncommon (figs. 62, 63).

HOLOZONIA Greene

The sole species, *H. filipes* (H. & A.) Greene, is a low perennial herb. The only collection available, *13083*, came from Boyes Springs, Sonoma County. Of this, several individuals were examined. The somatic chromosomes, totaling 28, are very small, botuliform, and of even contour (fig. 64). Trabants were not definitely identifiable. The 14 meiotic chromosomes, at the first metaphase, are perhaps the smallest in the tribe (fig. 65).

LAGOPHYLLA Nuttall

Included in the genus are three slender annual species, all of which have been investigated.

L. dichotoma Benth. n–7, 2n–14.—In the single set of plants studied, *13158*, from the San Benito Valley, three different individuals were wholly consistent in cytological details. In the somatic set (fig. 66) the most striking morphological feature is the very pronounced median constriction of a single pair, each of whose lengths is longer than that of any others. In addition, three other pairs possess more or less pronounced submedian constrictions; the shorter arm is usually bent around to give the chromosome a J-shape. The pair bearing the large, slightly elongated trabants are distinctly the shortest in the set. The meiotic chromosomes (fig. 67), which are large in consonance with the large size of the somatic set, are regularly constricted and display no recognizable morphological differences.

L. glandulosa Gray. n–7, 2n–14.—With this species, as with the preceding one, only one collection was available, *Keck 1348*, from 2 miles southwest of Yosemite Junction. Five plants were examined and all were essentially identical cytologically. The somatic chromosomes exhibited no morphological peculiarities (fig. 68), in direct contrast to those possessed by *L. dichotoma*. Two fairly large trabants are regularly present, although one is obscured in the figure.

The meiotic chromosomes are large and indistinguishable from one another (fig. 69). The microsporocytes are comparatively small.

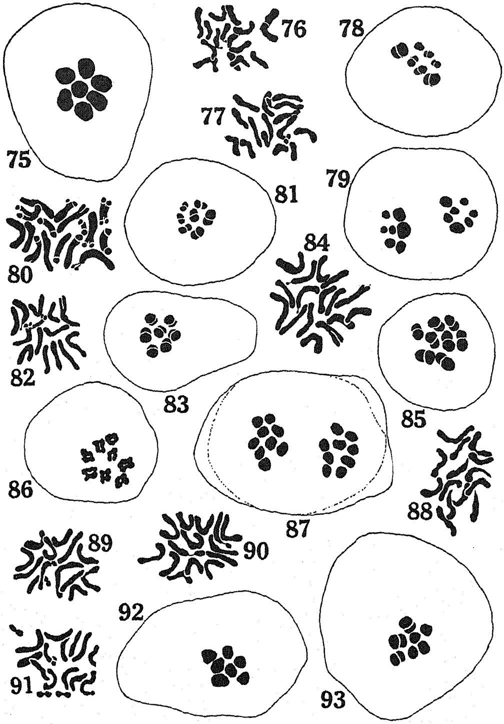
L. ramosissima Nutt. n–7, 2n–14, with fragmentation.—The following collections have been investigated: in 1931, *12910*, *12980;* in 1932, *Keck 1323*, *13093*, and *DAJ 690*. These covered a wide range of territory, extending from Grants Pass, Oregon, to Tehachapi Pass in southern California. Morphologically the somatic chromosomes are decidedly irregular in outline, being unevenly thickened. In the majority of the plates constrictions are difficult to identify (fig. 72), but in some plants of *Keck 1323* a rather high proportion of the chromosomes possess deep median to subterminal constrictions; at such places the chromosomes are often curved or bent abruptly. This species is noteworthy for the all but universal occurrence of fragmentation in its somatic chromosomes; there was hardly a plant which did not show more or less evidence of this phenomenon. It was in consequence possible to follow out all the details of the process, from the beginning of the constriction within the pellicle, with the two segments either sharply detached or connected together by a fine thread, until the final rupture of the pellicle and complete separation (figs. 70, 71). The length of the segmented portion is somewhat variable, generally being a trifle over a third of the whole length of the original chromosome. The total number of chromosomes in any one plate which may fragment is not known, but probably does not exceed four. Fusion (or perhaps merely mechanical cohesion) apparently occurs also, because of the counting of not more than twelve chromosomes on at least four occasions. In contrast to the other species of *Lagophylla*, the trabants of *L. ramosissima* are very tiny and attached at the ends of long, slender threads. The haploid chromosome number does not vary, and there is little or no morphological individuality, but it is to be noted that some of the bivalents have two chiasmata (for example, figs. 73, 74).

LAYIA H. & A.

The genus consists of approximately 15 species, of which eight have been studied. The species are all annuals, blooming in early spring. There is too close a resemblance between the species for them to be arranged in sections.

Layia jonesii Gray. n–7, 2n–14, with some evidence of fragmentation.—This species, *13144*, is found along the coast near Morro. Four individuals were examined. In most of the root tips, satisfactory plates were exceedingly rare, and even where the chromosomes were clearly separated, they were usually in a condition unsuited for morphological study. The chromosomes in one plant were distinctly smaller and more rounded up than in the other three. It appears that there is at least one pair with nearly median, and two pairs with subterminal, constrictions. The trabants are sometimes conspicuous. In practically every plate in one of the plants, a single chromosome bears a long constriction reminiscent of the type common in *Crepis*. In a few of the plates of this same plant, there was some evidence of fragmentation (or at least of the presence of more than 14 chromosomes), but as it was not observed in the other plants, it is difficult to say whether this phenomenon is as prevalent in this new species as it is, for example, in *L. platyglossa*. The meiotic chromosomes at the first metaphase are large, but apart from this nothing peculiar has been observed (fig. 75).

L. calliglossa (H. & A.) Gray. n–7, 2n–14, with occasional fragmentation.—Two forms, *13081* from El Verano, Sonoma County, and *13084* from Napa County, were studied intensively. Although on the whole the two forms possess the same number of chromosomes, there is a recognizable cytological difference between them in so far as the somatic chromosomes are concerned. In the somatic plates of *13081* (fig. 76) are to be found two large chromosomes lying on the periphery of the plate and with sharp median constrictions, two which are probably medianly constricted, four submedianly, and two short ones terminally, the last carrying tiny trabants. The set from *13084* (fig. 77) likewise shows two large chromosomes, but these are subterminally long-constricted; there are also two with median and four with submedian constrictions. Two chromosomes are much shorter than the others, but there are no trabants similar to those in *13081*. The differences between the somatic chromosomes in the two groups of plants therefore reside in the place and nature of the constrictions in the two large chromosomes and in the presence of inconspicuous trabants in one group. Ignoring these characteristics, the somatic chromosomes may be grouped into the following



FIGS. 75–93.—*Layia. L. jonesii:* fig. 75, MI (n-7). *L. calliglossa:* fig. 76, SM (2n 14); fig. 77, SM (2n-14). Fig. 76 is from *13081*, fig. 77 from *13084*, fig. 78, MI (n-7) from *13084*, and fig. 79, MII (n-7) from *13081*. *L. carnosa:* fig. 80, SM (2n-16). *L. chrysanthemoides:* fig. 81, MI (n-7). *L. elegans:* fig. 82, SM (2n-14); fig. 83, MI (N-7). *L. gaillardioides:* fig. 84, SM (2n-16); fig. 85, MI (n-8). *L. hieracioides:* fig. 86, MI (n-8). *L. nutans:* fig. 87, MII (n-9). *L. platyglossa:* fig. 88, SM (2n-14) from *13053*. Figs. 89–91 are from *13065*, figs. 89 and 90 are without fragmentation, while fig. 91 shows 2n-13 plus 3 pieces; fig. 92 (*13110*) and fig. 93 (*13152*), MI (n-7). ×2200.

pairs: two large, two fairly large, two small, and one very small. In one plant of *13081* there were a number of clear-cut cases in which 15 and 16 chromosomes were counted. The supernumerary elements were plainly fragments, hence it seems probable that one or both of the large medianly constricted chromosomes may at times become disjoined. In still another plant, 17 chromosomes were counted six times, and 18 twice, which might indicate that chromosomes other than the two large ones may also divide transversely.

In the two groups of plants, however, the behavior at meiosis is identical and reflects to a remarkable extent the differences at mitosis (figs. 78, 79). These are best revealed at the second metaphase; the sizes given for the somatic set are exactly duplicated for the gametic chromosomes (fig. 79, especially in the group at the left).

L. carnosa T. & G. 2n–16.—The plants were grown from seeds taken from vouchers collected by the writer in 1931 on sand dunes between Samoa and Rolph. The somatic chromosomes (fig. 80) are much alike and the longitudinal split appears in late prophase. The chromosomes are considerably curved, but many appear to have approximately median constrictions. In most of the plates it was impossible to locate the trabants; in the complement illustrated, a single chromosome bore two trabants.

L. chrysanthemoides (DC.) Gray. n–7.—Buds were obtained from plants growing along the Skyline Boulevard at the junction with the side road leading to the Broadway section of Burlingame. The meiotic chromosomes (fig. 81) are sharply differentiated by their sizes: there are two large, three medium, and two small bivalent chromosomes.

L. elegans (Nutt.) T. & G. n–7, 2n–14.—The sole set of plants studied, *13052*, was found near Etiwanda. There are some slight differences between the somatic chromosomes (fig. 82). The two trabant-bearing chromosomes are the bulkiest, but in most of the plates one cannot identify these two with entire certainty because the trabants are so tiny as to be unrecognizable in the rather dense cytoplasm surrounding the chromosomes. One pair is certainly constricted at the center while three other pairs are submedianly constricted. In the microsporocytes the chromosomes are unusually

clear at every stage; there are no differences distinguishing them from one another (fig. 83).

L. gaillardioides H. & A. n–8, 2n–16.—Two groups of plants were examined: *13109* from north of Tomales, and *DAJ 685* from along the road leading to La Honda from the Skyline Boulevard. There is little to distinguish either the somatic or the meiotic chromosomes from one another (figs. 84, 85). Constrictions were shown only vaguely in the somatic chromosomes and those seen appeared to be submedian. The tiny trabants are always plainly visible. Meiosis in *DAJ 685* is peculiar in that at the first metaphase and first anaphase a bivalent with two chiasmata was observed in all suitable cells.

L. hieracioides (DC.) H. & A. n–8.—The material was collected on a dry hill-side above Searsville Lake on the Stanford campus, at the same locality as was *Madia exigua.* The chromosomes at the metaphase of the first division appear to possess two chiasmata; they have never been seen in the rounded-up condition (fig. 86).

L. nutans (Greene) Jepson. n–9, 2n–18.—The only collection available, *13087*, was originally found on the Napa Range. The root tips were in such poor condition that exact computations of the chromosome number could be made in only a few cases. Nothing could be made of the structure of the somatic chromosomes, although these seem to be similar to one another structurally. The plates at metaphase of the first meiotic mitosis were unsatisfactory, but those at the second metaphase allowed no doubt concerning the gametic number. The meiotic chromosomes are all of the same size and configuration (fig. 87).

L. platyglossa (F. & M.) Gray. n–7, 2n–14, with fragmentation.—The following groups of plants were investigated: *13053* from the San Bernardino plains, *13065* from Uvas, Santa Clara County, *13110* from a point a mile south of Tomales, and *13120* on the 17-Mile Drive at Point Joe.

The behavior of the somatic chromosomes is so bewildering as to render the formulation of a comprehensive, logical diagnosis very difficult. As a matter of fact, it would appear that a separate description of each set would be necessitated, and that this could be prolonged more or less indefinitely. Consequently only four metaphase plates are being presented: fig. 88 is from *13053*, while figs. 89–91 are from the same root tip of a plant from *13065*. In the first

plate the absence of trabants will be noted. In fig. 89 there are two chromosomes with median, six with submedian, and two with abrupt terminal constrictions, while the other four elements are more or less straight. In fig. 91 most of the chromosomes are straight or slightly curved, lacking distinct constrictions. Two are apparently terminally constricted. One chromosome (in the lower right corner) possesses a subterminal constriction at each end. The three small pieces are probably extra chromosomes rather than fragments, since each is terminally constricted. The chromosomes in fig. 90 are more nearly of the same size amongst themselves than those in the other plates. In other plates there were several counts of 15, and five of 16 chromosomes; and in addition to these supernumerary elements there was other evidence of fragmentation.

Culture *13110* showed scarcely any evidence of fragmentation; *13120* showed some signs, but the differences in size and shape of the chromosomes were most pronounced in this group. Satisfactory plates were plentiful; most of them contained 14 chromosomes, while one gave 13 plus two small pieces and another the same number plus three fragments.

Meiosis is apparently as stable as mitosis is not. In practically every first metaphase plate were observed three large bivalents and four small ones (fig. 92); the larger chromosomes separate precociously (fig. 93), but distribution of all chromosomes is perfectly regular.

MADIA Molina

The genus consists of perhaps 18 species, the majority of which have been available for examination. As commonly treated, the genus is divided rather unsatisfactorily into four sections: Eumadia, Anisocarpus, Harpaecarpus, and Madaria.

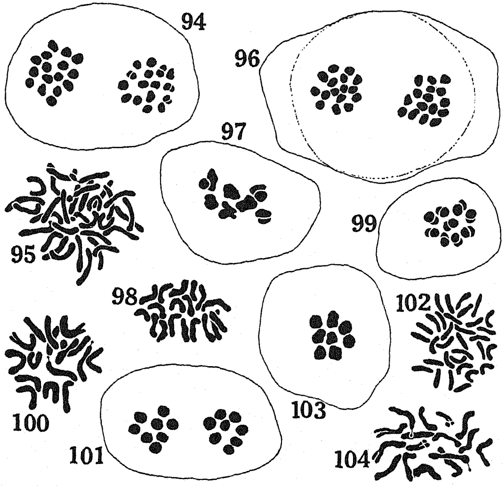
Section "ANISOCARPUS" (Nutt.)

The section, the largest one, contains seven species formerly included in the genus *Anisocarpus* Nutt. Three species were examined, those not available including *M. bolanderi* Gray, *M. valida* Brandegee, *M. rammii* Greene, and *M. radiata* Kellogg.

M. madioides (Nutt.) Greene. n–7.—The material, *DAJ 680*, was collected in woods on the old Woodside-Kings Mountain grade, San Mateo County. Despite the early separation of the chromo-

somes at the first metaphase, they are situated so far apart that counts are easy (fig. 97).

M. "*pumila*," an undescribed species. n–9, 2n–18.—The plants which were studied came originally from south of Knoxville. The somatic chromosomes are rather small, crowded, and tend to pair off. The contour is somewhat wavy, which complicates the identi-



FIGS. 94–104.—*Madia. M. dissitiflora:* fig. 94, MII (n-16). *M. sativa* ssp. "*congesta*": fig. 95, SM (2n-32); fig. 96, MII (n-16). *M. madioides:* fig. 97, MI (n-7). *M.* "*pumila*": fig. 98, SM (2n-18); fig. 99, MI (n-9). *M. yosemitana:* fig. 100, SM (2n-16); fig. 101, MII (n-8). *M. exigua:* fig. 102, SM (2n-32). *M. elegans:* fig. 103. MI (n-8); fig. 104, SM (2n-16). ×2200.

fication of constrictions, which certainly seem to exist. No trabants could be found (fig. 98). The meiotic chromosomes at the first metaphase, as in most of the other species, disjoin early (fig. 99), but are so well separated that counts are thereby facilitated.

M. yosemitana Parry. n–8, 2n–16.—A single group of plants,

13186, from the Hetch-Hetchy Road, provided the material. The somatic chromosomes are bulky, although two pairs are a trifle narrower than the others; some show definite evidence of a median, others of a submedian or subterminal, constriction (fig. 100). Two small trabants occur. The second metaphase configurations (fig. 101) are more satisfactory than those at the first metaphase and were found in abundance.

Section EUMADIA Gray

The section contains five species, of which two have not been available, *M. citriodora* (Gray) Greene and *M. glomerata* Hook.

M. anomala Greene. n–16, 2n–32.—A single collection, *13074*, from Mill Valley, was studied. The root tips and buds were not at all in the best condition, but the accuracy of the counts seems sure.

M. dissitiflora T. & G. n–16, 2n–32.—The following collections have been examined: *13075* from Mill Valley, *13091* from the Napa Range, *13855* from north of Jackson, *DAJ 684* from La Honda, and *DAJ 681* from Grant's Ranch, Mt. Hamilton. The root tips of this species are difficult to manipulate; there are exceedingly few clear plates. The tips were examined before the buds were available and the somatic number was presumed to be 30, owing to the fact that the chromosomes are so prevailingly interlaced and twisted that a positive count was difficult to make. However, after finding many clear first and second metaphase plates (fig. 94), which showed the haploid number to be 16, the root tips were re-examined. There now seems to be little question of the somatic number being other than 32.

M. sativa Molina. 2n–32.—The form studied, *12880*, came from Crow Creek Road, Alameda County. There were but few countable figures in the several root tips examined, but the somatic number is certainly 32.

In addition to the typical *M. sativa*, one subspecies may be recognized. This is the variety *congesta* of TORREY and GRAY. Three collections representative of this form were examined: *13078* from the Gallinas Valley, *13080* from the Sonoma Valley, and 13146 from San Simeon. The cells of the root tips are so small and the chromosomes so numerous that very few clear plates are to be encountered.

In these, 32 chromosomes were seen. Although in most of the plates the chromosomes are without distinguishing features, in root tips of *13080* some chromosomes were seen to have median, others subterminal constrictions. Two rather large trabants are present (fig. 95). The meiotic plates at the second metaphase are clear and show the haploid number to be 16 (fig. 96); the chromosomes at the first metaphase are too much crowded together and disjoin so early that it is difficult to estimate the number accurately.

Section HARPAECARPUS Gray

The section includes only the following species.

M. exigua (Sm.) Greene. 2n–32.—The material was collected from plants growing on a hill-side above Searsville Lake on the Portola-La Honda Road, and from others originally from Muir Gorge (the first collection by the writer, the other by HALL). The somatic chromosomes are small and essentially similar in structure throughout (fig. 102). Trabants were not definitely identified.

Section MADARIA Gray

Included in the section are six species of such close affinity that probably all could be included within the Linnaean species *Madia elegans* Don. The material available for cytological examination would be classified clearly as of that species.

M. elegans Don. n–8, 2n–16.—Plants from six localities were examined: *12867* from Grass Valley, *13020* from Cuyamaca Lake, *13163* from Ackerson Meadow, *13195* from the Indians, Santa Lucia Mts., *13196* from Santa Lucia Peak, and *Keck 1279* from Crystal Springs Lake. A considerable range of territory is represented, although *13195* and *13196* were close together but differed in that the latter is a dwarf form. All six forms were identical in all their cytological aspects, with the possible exception of inconsequential minor morphological variations. The somatic chromosomes are comparatively small, irregularly thickened and contorted, and the presence of constrictions is thus rendered problematical (fig. 104). The trabants are on long, slender threads; this characteristic is more pronounced in *13163* than in the other forms. In decided contrast to the other species in *Madia*, the first metaphase meiotic chromosomes do not separate until late in the stage, but are rounded up and clearly separated (fig. 103).

Summary

In the following list are tabulated the results of the study.

Species	Examined: Collections	Examined: Plants	n	2n	Remarks
Achyrachaena mollis	3	∞	8	16	No trabants (?)
Hemizonella minima.........	1	1	ca. 13	ca. 26	
Hemizonia					
Sect. Blepharizonia					
H. plumosa	1	1	14		
Sect. Calycadenia					
H. bicolor	4	8	6	12	
H. ciliosa	4	6	6	12	
H. mollis	4	5	7	14	No trabants (?)
H. pauciflora	1	1	5	10	
H. truncata	4	5	7	14	Meiotic irregularities
ssp. "scabrella"	3	9	7	14	
H. villosa	1	1	7	14	
Sect. Centromadia					
H. congdoni	4	4	......	24	
H. parryi	2	2	......	24	One trabant
H. pungens	11	14	9	18	Meiotic irregularities
ssp. "maritima"	3	3	9	18	Fragmentation
Sect. Euhemizonia					
H. congesta ssp.:					
calyculata	1	4	14	28	
clevelandi	1	1	14	......	
lutescens	1	2	14	28	
luzulaefolia	4	7	14	28	
typica	3	3	14	28	Fusion, fragmentation
H. wheeleri	1	1	8	......	
Sect. "Fruticosi"					
H. clementina	2	2	12	24	
H. greeneana	1	6	12	24	
Sect. Hartmannia (pars)					
H. angustifolia	22	∞	10	20	No trabants (?)
ssp. "macrocephala"	3	3	10	20	
H. fasciculata ssp.:					
"ramosissima"	8	8	12	24	
"superfasciculata"	1	1	......	24	One trabant
"lobbi" (Form 1)	2	2	14	......	
"lobbi" (Form 2)	1	1	......	22	
"hispidula"	3	3	......	22	Trabants sometimes lacking
H. floribunda	1	2	13	26	
H. kelloggii	1	1	9	18	Meiotic irregularities
H. "pallida"	1	∞	9	......	
H. paniculata	24	29	12	24	Fragmentation
ssp. "increscens"	1	1	......	24	
{H. virgata	4	5	4	8	
{H. heermanni	5	5	6	12	Meiotic irregularities
Holozonia filipes	1	4	14	28	No trabants (?)

Species	Examined: Collections	Examined: Plants	n	2n	Remarks
Lagophylla					
L. dichotoma	1	3	7	14	
L. glandulosa	1	5	7	14	
L. ramosissima	5	∞	7	14	Fragmentation
Layia					
L. calliglossa	2	7	7	14	Fragmentation; size differences
L. carnosa	1	5	......	16	
L. chrysanthemoides	1	∞	7	......	Size differences
L. elegans	1	2	7	14	
L. gaillardioides	2	3	8	16	
L. hieracioides	1	∞	8	......	
L. jonesii	1	4	7	14	Fragmentation
L. nutans	1	2	9	18	
L. platyglossa	4	12	7	14	Fragmentation
Madia					
Sect. Anisocarpus					
M. madioides	1	∞	7	......	
M. "pumila"	1	∞	9	18	No trabants
M. yosemitana	1	2	8	16	
Sect. Eumadia					
M. anomala	1	1	16	32	
M. dissitiflora	5	∞	16	32	
M. sativa	1	1	......	32	
ssp. "congesta"	3	3	16	32	
Sect. Harpaecarpus					
M. exigua	2	∞	......	32	No trabants (?)
Sect. Madaria					
M. elegans	6	8	8	16	

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HISTOLOGICAL AND REGENERATIVE STUDIES ON THE FLAX SEEDLING

CONTRIBUTIONS FROM THE HULL BOTANICAL LABORATORY 446

DONALD M. CROOKS

(WITH FORTY-FOUR FIGURES)

Introduction

Common flax, *Linum usitatissimum* L., is the only member of the Linaceae of economic importance (6, 11). The fibers are a source of linen, the seed is a commercial source of linseed oil, and the residue after the oil is pressed from the seeds is an important source of food for livestock. Most of the early studies of the plant were made in Europe (4, 5, 6, 7, 8, 15, 16, 17, 18). Critical investigations of all phases of fiber development and of the commercial use of flax have been carried on in Holland, Germany, Russia, and in the United States by the Department of Agriculture.

Histological studies on the seedling were confined almost entirely to Europe during the last quarter of the 19th century. GÉRARD (5) and TOGNINI (15) reported investigations of the transition from root to stem; JANCZEWSKI (7) classified the histogens of the primary root; VAN TIEGHEM (17) described the origin of the lateral roots; and WILDE (18) described transverse sections of the mature root, stem, and leaf. TSCHIRCH and OESTERLE (16) described the fruit, reported large aleurone bodies and no starch in the cotyledons, and gave a brief description of germination. In most of these studies flax was selected merely for morphological comparison as a representative of the Linaceae. Certain phases of vegetative regeneration of shoots were observed by REICHARDT (12). His observations were verified by TAMMES (13) in Holland, and the histology of the regenerated parts was investigated by BEALS (3) in the United States. HERZOG (6) deals with the botanical, cultural, and commercial aspects of the plant. Extensive bibliographical lists are given by HERZOG (6) and TAMMES (13). The literature citations included in this paper are

limited to those directly concerned with some phase of seedling histology. The present paper supplements the studies previously made on the histology, anatomy, and regeneration of flax seedlings, and deals in particular with the ontogeny of tissues and organs.

The Bison variety of flax, which is widely grown in North and South Dakota, was used in this investigation. Plants were grown in the greenhouse at the University of Chicago during the winter of 1931–32 and compared with material grown in gardens during the following summer at Ball State Teachers College, Muncie, Indiana. Material from these sources showed no morphological differences except a slightly longer axis in some of the greenhouse plants.

Various fixatives were used. The most satisfactory results were secured with a modification of Nawaschin's solution which consists of solution A (7 cc. glacial acetic acid, 1 gm. chromic acid crystals, and 92 cc. distilled water) and solution B (30 cc. formalin and 70 cc. water) mixed in equal volumes just before using. A solution made of 5 cc. of formalin, 5 cc. of glacial acetic acid, and 90 cc. of 50 per cent alcohol gave fair results except when it was desired to secure mitotic figures and a minimum shrinkage of the protoplasm. The greatest number of division figures were obtained in roots killed at 11 P.M. and in stem tips and leaves killed at 1 P.M. Chloroform was used for clearing. Final infiltration of paraffin was accomplished very slowly, in two stages: (1) by evaporating the chloroform from the paraffin-chloroform mixture in an evaporating dish placed on top of the oven for a period of about 48 hours; and (2) by transferring to an oven at 55° C. until no taste of chloroform remained.

Serial sections were cut 6–15 μ in thickness and stained in a modification of Flemming's triple stain. All drawings (except figure 3) were made by the use of a microprojection apparatus.

Germination and young seedling

Under greenhouse conditions seeds germinated rapidly at a temperature of 65°–90° F. At a temperature of 65°–70° F. the cotyledons showed above the ground in four or five days, and the seedlings appeared much the same as summer-grown plants. Higher temperatures gave more rapid germination, but after a few days the hypocotyl of the seedlings elongated greatly, as compared with those

grown outside during the summer or in cooler temperatures in the greenhouse.

In the process of germination the primary root emerges from the seed coat by the end of the first or second day and grows downward, thus forming a type of tap root which becomes the axis of the entire root system (2). Before the cotyledons show above the surface of the soil the hypocotyl region, in response to elongation in the basal portion, bends to the shape of an inverted U, so that the seed coat and cotyledons are pulled above the ground (10). Sometimes the cotyledons are pulled out of the seed coat while yet in the soil. The hypocotyl straightens out in about five days and the remainder of the seed coats is pushed off by the enlarging cotyledons. After a period of general growth of the hypocotyl, the first major zone of elongation is at the ground level. This zone of elongation progresses upward along the hypocotyl to the cotyledons, ceasing at the upper level in about 15–20 days. In five or six days several lateral roots appear on the primary root and are usually formed within 3 cm. of the surface of the soil. Most of the lateral roots originate from the upper 8 cm. of the primary root.

As the cotyledons push from the seed coat, they spread apart and continue to expand for several days before the epicotyl begins to elongate. The cotyledons are obovate and almost sessile, and become true photosynthetic leaves. The epicotyl grows very slowly at first. By 12–15 days it appears as a compact bud of miniature leaves with almost no elongation of internodes. After about 20 days the elongation of internodes causes very rapid growth in height. At maturity the first internode is about 1 mm. long, the second about 15 mm., and all others are usually from 5 to 10 mm. Most of the leaves are simple and sessile, although several plants have one or two double leaves between the fifth and tenth node (fig. 25).

Primary root

The primary root is diarch, with three to five well defined protoxylem elements alternating with two rather large groups of small primary phloem cells (fig. 15). The metaxylem elements are successively larger toward the center. The pericycle, which is differentiated early, is a single layer of cells. The Casparian strips of the

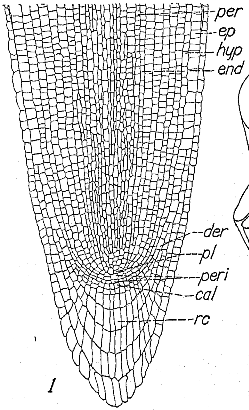
endodermis never become very thick but are deposited on the radial walls before the metaxylem is completely differentiated. The cortical cells are very regular with large intercellular spaces. The outer layer of the cortex forms a definite hypodermis which appears much as the epidermis, except that none of the cells elongate and become root hairs. The cortex persists after considerable secondary thickening, and the first breakdown of cortical cells occurs in the region midway between the endodermis and hypodermis.

Development of the root axis is by clearly defined histogens (fig. 1) which are similar to those described by JANCZEWSKI (7). The plerome and periblem give rise to the stele and cortex respectively; while a layer of cells, which makes up the calyptrogen and dermatogen overlying the periblem, produces the epidermis and the root cap.

The calyptrogen-dermatogen layer of cells overlying the periblem multiplies by periclinal divisions around the tip and forms a root cap of regular, radial rows of cells. In the lateral portion of the same layer the epidermis is formed by anticlinal divisions. Laterally, where the divisions are anticlinal the histogen is strictly a dermatogen, while the layer at the tip is a calyptrogen. The root cap at the tip is very regular because the cell divisions in the calyptrogen are periclinal only. Occasionally some of the cells in the lateral portion of the root cap divide anticlinally and distort the radial rows. This occurs only in lateral portions of the root cap and not at the tip, where the rows of cells are regular.

The cortex is derived from the periblem, which consists of two layers of cells overlying the plerome. The outermost layer of the periblem divides only anticlinally and forms the outer layer of the cortex, the hypodermis. The inner layer divides in all planes and forms the remainder of the cortex, which at maturity is about 6–9 cells in thickness (figs. 1, 2).

The stele is differentiated from a small group of plerome cells which divide in all planes at the tip, while divisions at a higher level are chiefly in a transverse plane. The outer layer of this group produces the pericycle, which can be distinguished rather early by the relatively dense cytoplasm and larger size of the cells. The first evidence of differentiation of the vascular system appears about 0.4 mm. above the tip of the plerome. Two primary phloem ducts are



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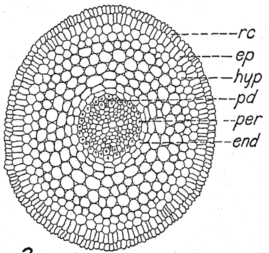

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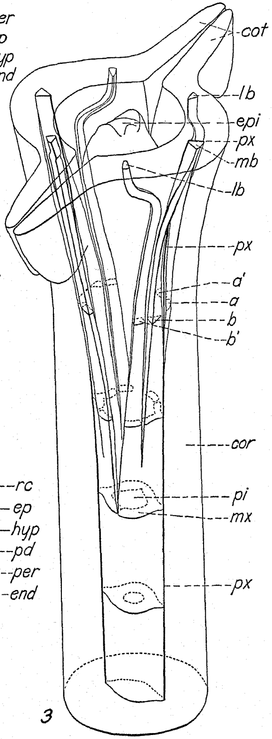

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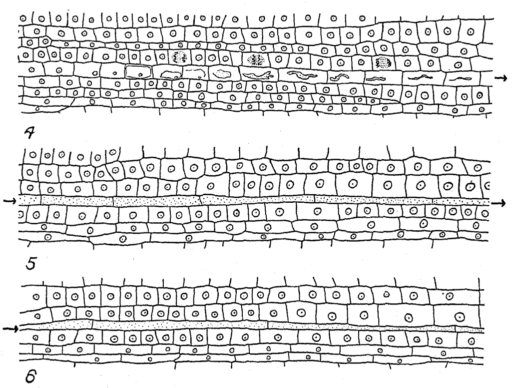
FIGS. 1–3.—Fig. 1, median longitudinal section of primary root: *per*, pericycle; *ep*, epidermis; *hyp*, hypodermis; *end*, endodermis; *der*, dermatogen; *pl*, plerome; *peri*, periblem; *cal*, calyptrogen; *rc*, root cap. Fig. 2, transverse section 0.7 mm. from tip of primary root: *pd*, primary phloem duct.

Fig. 3, schematic diagram of vascular system of hypocotyl showing transition from root to cotyledons: *cot*, basal portion of cotyledons; *lb*, lateral bundle of cotyledon; *epi*, epicotyl; *mb*, median double bundle of cotyledon; *px*, protoxylem; *mx*, metaxylem; *pi*, pith; *cor*, cortex; *a*, *b*, bundles which anastomose and form double bundle of cotyledon; *a′*, *b′*, lateral metaxylem elements which form lateral bundles of cotyledon.

first differentiated opposite each other by the elongation and breakdown of a single row of cells lying next to the pericycle, and alternate with the two protoxylem points which are differentiated later. Figures 4, 5, and 6 show how these ducts are formed and stretched by the elongation of neighboring cells. These ducts collapse after becoming greatly stretched but usually persist until the protoxylem elements are well differentiated. Elongation of the root often causes



FIGS. 4–6.—Longitudinal sections of primary root showing formation of primary phloem duct at 0.6, 1, and 1.5 mm. from tip.

the protoxylem to collapse. The metaxylem elements differentiate centripetally until all the elements of the xylem plate are lignified and no central parenchyma remains. The outermost metaxylem elements have scalariform wall thickenings while those centrally located have pitted wall thickenings.

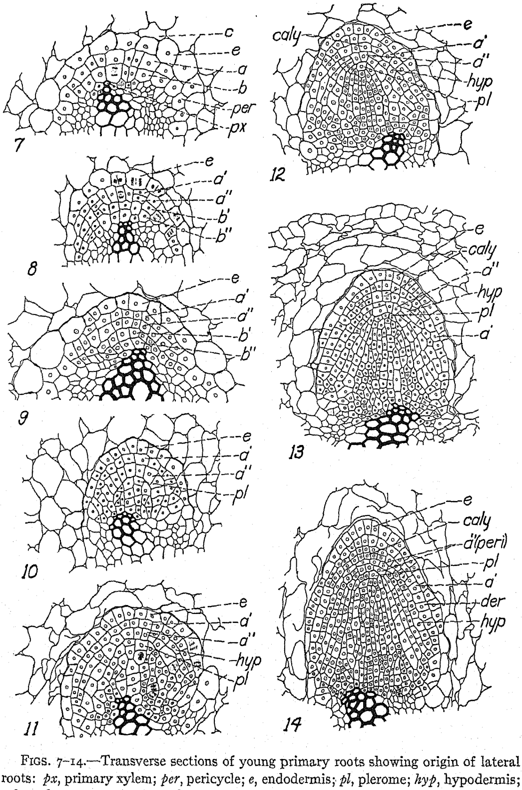
Lateral root histogens

Lateral roots are formed in the ontogeny of the primary axis about the time the thickenings are deposited on the walls of the metaxylem cells. Several lateral roots develop just below the soil level and are usually oriented slightly to one side of the protoxylem point, but

may occur on the same radius directly outside this point (9). Development is initiated by a tangential division of several cells of the pericycle next to a protoxylem point (fig. 7). This division produces two distinct layers (*a*, *b*), each of which in turn again divides by another tangential division (figs. 8, 9). The inner layer (*b*) usually divides somewhat in advance of the outer layer (*a*), as is indicated in figure 8. Figure 9 shows the second tangential division completed, forming four layers of cells, the outer two (a', a'') being derivatives of (*a*) and the inner two (b', b'') being derivatives of (*b*). A few radial divisions which compensate for the increase in length of overlapping layers occur in the two- and four-layered pericycle, but these divisions usually cause no distortion of the tangential rows. The outer (a') of the four layers derived from the pericycle gives rise to the calyptrogen and dermatogen; the next (a'') gives rise to the periblem; and the two inner ones (b', b'') continue cell activity as a plerome.

By the time four layers of cells have been derived from the pericycle, the endodermis becomes active by radial divisions (9, 17). At this stage the endodermis can easily be identified by the Casparian strips which persist on the radial walls after several subsequent radial divisions in the original cells. The endodermis continues to divide in a plane anticlinal to the developing lateral root, and forms a uniseriate layer of cells about the tip. This layer produced from the endodermis never becomes more than one cell in thickness. Cell activity continues until the lateral root has pushed through the cortex of the primary root into the soil and the cell layer derived from the endodermis is sloughed off with the outer layer of the root cap.

The four layers of cells derived from the pericycle establish the first histogen of the new lateral root. The inner two layers (b', b'') continue to divide in all planes and form a conical group of cells, the plerome (fig. 10). Several subsequent divisions follow in the plerome before any tangential divisions take place (periclinal to the new lateral root) in the outer two layers (a', a''). All subsequent development and differentiation of the plerome is similar to that described for the primary root. The primary xylem of the lateral root is diarch, and the protoxylem elements lie in the same plane as those of the primary root from which the lateral root originates.



FIGS. 7–14.—Transverse sections of young primary roots showing origin of lateral roots: *px*, primary xylem; *per*, pericycle; *e*, endodermis; *pl*, plerome; *hyp*, hypodermis; *caly*, calyptrogen; *peri*, periblem; *der*, dermatogen; *a*, outer layer of first two layers derived from pericycle; *b*, inner layer of first two layers derived from pericycle; *a'*, outer layer derived from *a*; *a''*, inner layer derived from *a*; *b'*, outer layer derived from *b*; *b''*, inner layer derived from *b*. Derivatives of layer *a'* become the calyptrogen and dermatogen; derivatives of layer *a''* become the periblem; derivatives of layer *b* become the plerome.

Concurrent with increase in the size of the plerome, several radial divisions (anticlinal to the new lateral root) occur in the two outer layers from the pericycle (a', a''). Following establishment of the plerome, the innermost (a'') of the two outer layers begins periclinal divisions at a point farthest from the tip (fig. 11). The periclinal divisions in this layer (a'') occur successively toward the tip for a time and then their innermost derivatives undergo periclinal divisions. This condition is shown on the right side of figure 11, where the basal part of layer a'' has divided once and the innermost derivative is again undergoing division, as indicated by a mitotic figure. The number of cell layers is increased by successive periclinal divisions of the newly formed innermost layers (fig. 12). Such periclinal divisions are continued progressively toward the tip of the new root, and finally the one to four remaining cells of layer a'' (fig. 13) are divided once periclinally, and the fundamental plan of the periblem (fig. 14) becomes established on the same plan as described for the primary root. The outer layer of the periblem divides only anticlinally, thereby continuing the uniseriate layer which differentiates into the hypodermis. At the tip of the root the innermost layer of the periblem continues to produce, by subsequent divisions, the remaining six to eight layers of the cortex (fig. 14).

By the time the layer a'' and its derivatives have developed to the extent that about three layers of cells are formed about the base of the conical plerome, the outermost layer (a') is divided at the tip by periclinal divisions (fig. 12). This initiates development of a calyptrogen by forming two layers of cells of which the outer is the root cap while the inner remains meristematic, functioning as a calyptrogen and later dividing to form the second layer of the root cap. By such subsequent periclinal divisions the root cap is produced and the calyptrogen is perpetuated as a single layer of meristematic cells overlying the periblem. The laterally oriented cells of the layer a' continue anticlinal divisions as a dermatogen, which is the same as was described for the primary root.

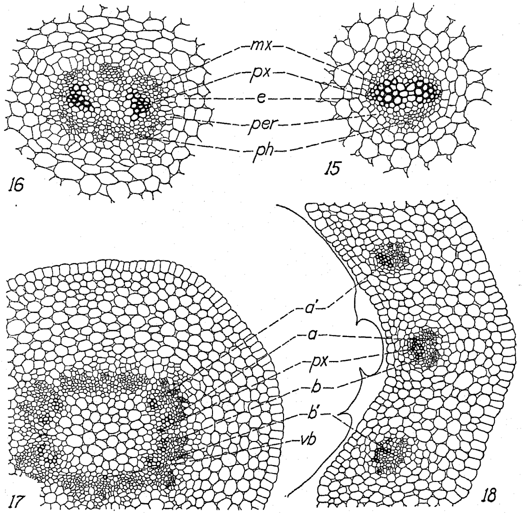
With all the histogens of the lateral root established on the same plan as the primary root, the subsequent growth and differentiation is also as that described for the primary root. There is little accumulation of cell débris or distortion of cortical cells about the tip

of the young lateral root as it grows through the cortex of the primary root. This would seem to indicate that there is some digestion as well as mechanical crushing of the cortical cells as the lateral root develops.

Hypocotyl, cotyledons, and transition

The lower portion of the hypocotyl is rootlike in vascular arrangement. A section made at approximately the ground level shows a primary xylem plate without central pith and two phloem groups alternate with the protoxylem elements (fig. 15). The central portion of the stele of the hypocotyl is parenchymatous at approximately one-fourth of its length above ground. There is a gradual reorientation of the primary vascular tissue at successively higher levels throughout the hypocotyl and basal part of the cotyledons. In the lower one-fourth of the hypocotyl the two phloem groups diverge and form four phloem regions which at a higher level are equally distributed at 90° intervals about a cross-section (fig. 16). Phloem which is associated with the first two leaves of the epicotyl is soon differentiated in the hypocotyl, and anastomoses at the point at which the phloem groups of the lower hypocotyl diverge. In the central region of the hypocotyl the metaxylem elements are differentiated in a tangential position with respect to the protoxylem points. This forms four metaxylem groups, each of which is accompanied by a corresponding phloem strand located outward on the same radius. Somewhat above the middle region of the hypocotyl, the four metaxylem groups with the related phloem groups are each differentiated as two groups, so that eight vascular strands associated with the cotyledons are formed. At successively higher levels throughout the hypocotyl the four metaxylem groups (fig. 3 *a*, *a*′, *b*, *b*′) related to each protoxylem point become oriented more and more definitely lateral to the protoxylem (figs. 3, 17, 18). In the upper region of the hypocotyl this orientation of the eight cotyledonary bundles results in two widely separated groups, each of which becomes a cotyledonary trace. At the point of divergence of a cotyledon the two lateral bundles of metaxylem and accompanying phloem (*a*′, *b*′) of each group of four become widely separated from the two middle bundles (*a*, *b*). Each of these lateral bundles (*a*′, *b*′) is a

bundle of the trace of a lateral vein of the cotyledon. At successively higher levels in the upper hypocotyl and basal part of the cotyledon,



FIGS. 15–18.—Transverse sections of seedling 6 days old showing transition from root to cotyledon. Fig. 15, transection about 1. mm. below ground level. Fig. 16, transection in middle region of hypocotyl showing parenchymatous center of stele and early breakdown of protoxylem. Fig. 17, transection of upper region of hypocotyl showing 8 metaxylem groups with associated phloem (also breakdown of protoxylem). Fig. 18, transection at base of cotyledons and epicotyl showing level where two metaxylem groups anastomose and showing also two phloem groups which retain their identity until a higher level in the cotyledon (two lateral veins of cotyledon also shown): *mx*, metaxylem; *px*, protoxylem; *e*, endodermis; *per*, pericycle; *ph*, phloem; *vb*, vascular bundle of first leaf; *a*, *b*, bundles which anastomose and form double bundle of cotyledon; *a′*, *b′*, lateral metaxylem bundles which form lateral bundles of cotyledon.

the two metaxylem groups (a, b) nearest the protoxylem become oriented more and more in an endarch relationship to the proto-

xylem. In the base of the cotyledon the two metaxylem groups are differentiated in an endarch relation to the protoxylem, and at this level the two groups of metaxylem anastomose (fig. 18) and form the median double bundle (fig. 26) of the cotyledon (14). The two phloem groups associated with the median bundle retain their identity for 2–3 mm. farther into the cotyledon and then anastomose (fig. 18).

Figure 3 shows the transition in a semidiagrammatic manner. Each strand shown in this diagram may be interpreted as a bundle of associated xylem and phloem. The vascular elements of the epicotyl are differentiated somewhat later than the vascular system related to the cotyledons. Figure 17 shows a zone of phloem which is associated with the first leaves. These bundles are differentiated as endarch, collateral bundles throughout the hypocotyl, where they may anastomose with the lateral arcs of metaxylem and accompanying phloem in the lower hypocotyl, or may end blindly in the parenchyma. The bundles related to the first two leaves are small in the middle and lower hypocotyl, and usually anastomose with the lateral arcs of metaxylem and accompanying phloem; while the other bundles related to the epicotyl end blindly and have vascular connection only through secondary tissue. The transition is actually a root cotyledon transition as there is no reorientation of bundles that have relationship to the epicotyl.

In order to follow the transition in the middle and upper hypocotyl, it is necessary to examine plants before the hypocotyl has greatly elongated. Plants six days old, or when the cotyledons first come above the soil, have well differentiated primary xylem throughout the hypocotyl and cotyledons. As the hypocotyl elongates the protoxylem is crushed and most of it is obliterated by digestion. Later the early metaxylem breaks down and finally nearly all of the primary xylem disappears in the middle and upper hypocotyl. All of the primary xylem elements in the upper region of the hypocotyl have spiral wall thickenings. This is the last region to cease elongation, and the consequent and relatively continuous stretching causes a collapse of the spiral vessels.

By the time plants are 13 days old, most of the primary xylem of the upper region of the hypocotyl is crushed and is partly resorbed (fig. 19). CHAUVEAUD (4) has reported breakdown and complete

resorption of primary xylem in *Mercurialis* and *Allium*, much the same as was observed in *Linum*. In *Linum* there are fragments of the primary xylem that remain after 30 days, and may remain through the life of the plant, without being completely resorbed. Many cross-sections through the upper hypocotyl show no protoxylem and very little metaxylem, while at other levels fragments of

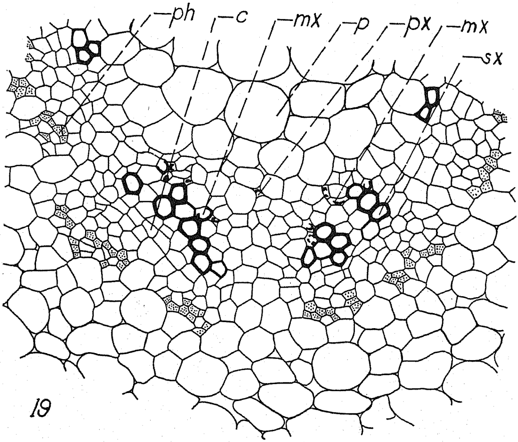


FIG. 19.—Transverse section in upper region of hypocotyl showing early breakdown and resorption of primary xylem: *c*, cambium; *mx*, metaxylem; *px*, protoxylem; *sx*, secondary xylem; *p*, pith; *ph*, phloem.

the elements can be found between the parenchyma cells. The ephemeral nature of the primary xylem probably led to some misinterpretation when GÉRARD (5) and TOGNINI (15) described the transition from root to stem in *Linum*. TOGNINI observed the breakdown of primary xylem but did not distinguish protoxylem and metaxylem in respect to their time and position of differentiation in the upper region of the hypocotyl.

The cotyledons are almost sessile, and in young seedlings they are

somewhat wider than the hypocotyl at their point of divergence. In the young seedlings they are made up of very compact storage cells.

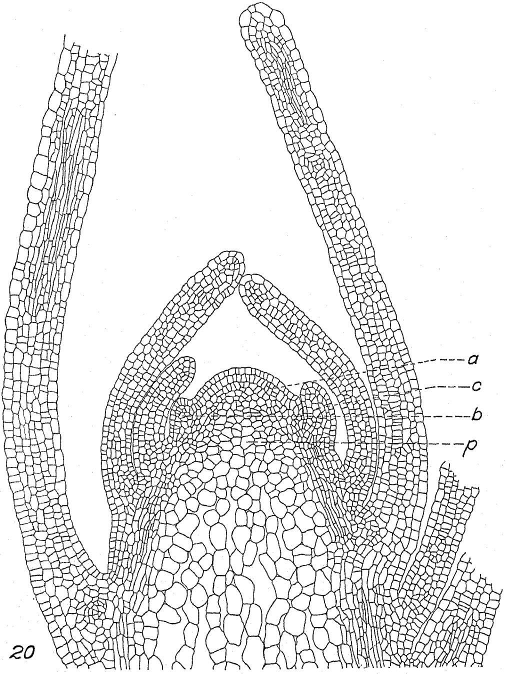


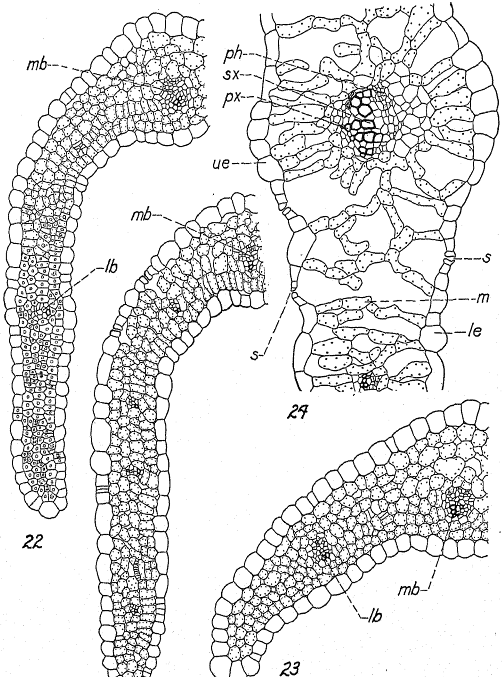
FIG. 20.—Median longitudinal section of growing point showing several stages of ontogeny of leaf: *a*, section of very young primordium; *b*, *c*, older primordia showing early development of provascular strands; *p*, pith.

Two or three of the adaxial layers take the form of a palisade layer, while the lower mesophyll consists of four or five layers of more

rounded, compact cells. Protoxylem elements are differentiated in the median bundle of the cotyledon in seeds placed in conditions favorable for germination for a period of 24 hours, and in some cases may be differentiated in the mature seed. Usually a few scattered phloem cells can be identified in the provascular strands of the young cotyledons before any wall deposits can be noted in the cells of the primary xylem. After the cotyledons are above the ground they continue to thicken and enlarge for a period of about 15 days. The photosynthetic tissue of the mature cotyledon has in general the structure of a mature leaf (fig. 24), except that it is considerably thicker and has smaller air spaces. Both the upper and lower epidermis have a considerable number of stomata with large air spaces underlying them. The closed, netted venation of the cotyledon and leaf is essentially the same (figs. 26, 27). Except in a short region in the median vein, the vascular bundles of the cotyledons are collateral and develop no secondary tissue, while the large leaf bundles develop considerable secondary xylem and phloem. The xylem elements of the cotyledons have spiral or loosely netted wall thickenings. After about 30 days the cotyledons become yellow and die.

Epicotyl and foliage leaves

The young epicotyledonary axis is surrounded by the bases of the cotyledons and develops very slowly for the first eight to ten days. The growing point cuts off leaf primordia which have an irregular phyllotaxy. The leaves at the first three nodes are usually in alternate pairs. Above the fourth node the leaves are arranged spirally, with one leaf at a node in a more or less indefinite phyllotaxy. Tognini (15) classified the arrangement of the upper leaves in a 2/5 system of phyllotaxy but states that frequently there are exceptions, even in different parts of the same plant. In this investigation no definite phyllotaxy could be established for any one plant, except for short intervals which suggested a 2/5 system. Occasionally three leaves are in a whorl at one of the lower nodes, and often the sixth, seventh, or eighth leaf is bilobed (fig. 25). A bilobed leaf may be broad and cleft almost to the base, or narrow with a very small cleft at the tip. The vascular system of such leaves shows them to have the form of a double leaf or of two leaves nondiverged in varying

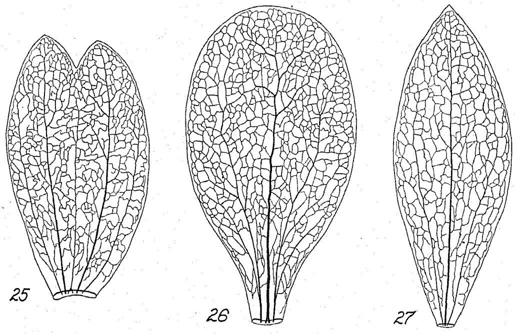


FIGS. 21–24.—Figs. 21–23, transverse sections of first leaf when its total length is 2.5 mm. Fig. 21, transection 0.9 mm. from tip of leaf where cell divisions have ceased. Fig. 22, transection 1.8 mm. from tip of same leaf showing region of meristematic activity in lamina. Fig. 23, transection 0.1 mm. from base of same leaf showing level where cell divisions have ceased: *mb*, median bundle; *lb*, lateral bundle.

Fig. 24, Transverse section through median vein of mature leaf from middle region of stem: *px*, primary xylem; *ph*, phloem; *sx*, secondary xylem; *s*, stoma; *le*, lower epidermis; *ue*, upper epidermis; *m*, mesophyll.

degrees. A double leaf usually has two perfect leaf traces. The lateral bundles of the two traces anastomose and form a small bundle which is median in the double leaf (fig. 25).

A leaf primordium arises from tissues of at least the three outermost cell layers of the growing point and forms a conical mass of cells. It elongates and broadens by general meristematic activity until it becomes a somewhat flattened, finger-like projection. The procambial strands are differentiated early (fig. 20 *c*). General meri-



FIGS. 25–27.—Diagrams showing venation of cotyledon and foliage leaves: fig. 25, double leaf; fig. 26, cotyledon; fig. 27, typical early foliage leaf.

stematic activity increases the length of a flattened primordium to about one-sixth or one-fifth the length of a mature leaf. Continued meristematic activity laterally and toward the adaxial surface results in formation of the lamina (fig. 22). During formation of the lamina other cells of the mesophyll undergo enlargement and further differentiation, and the vascular bundles are more completely differentiated. Meristematic activity first ceases in the tip of the leaf, next in the basal portion, and last in the basal portion of the lamina (figs. 21, 22, 23). After the young leaf has attained one-sixth to one-fifth its mature size all further expansion is by enlargement, stretching, and pulling apart of cells.

At maturity the mesophyll is very loose, with large intercellular spaces (fig. 24). There are about four cell layers of somewhat elongated cells which are essentially alike and not differentiated into a definite palisade and spongy parenchyma. In the mesophyll there is a complicated network of veins of various sizes. The median vein and sometimes the two lateral veins develop considerable secondary tissue. The smaller veins have fewer and fewer elements, and either form a closed netted system or may end blindly in the mesophyll. The epidermal cells are irregular in size and have a thin deposit of cutin on the outer walls. The stomata are numerous and occur in about equal numbers on both sides of the leaf. The guard cells are subtended by accessory cells.

The fundamental plan of the primary vascular system of the stem is differentiated early, in the form of procambial strands in the leaf primordia and growing point. The leaf primordia are close together, so that a length of 0.1–0.15 mm. of the growing point includes 10–20 nodes and internodes. The procambial strands are differentiated in the leaf primordia and to a distance of 0.1–0.15 mm. in the meristematic zone of the growing point at essentially the same time (fig. 20 *b*). Even in this short distance the strands extend through 10–20 nodes, and as the internodes elongate the vascular system is elongated with the same fundamental plan and with no differentiation of anastomosing bundles. The mature primary vascular system is then made up of foliar bundles, all of which are collateral. The leaf trace consists of three large bundles which differentiate as separate bundles in the cortex and anastomose at the point at which the trace becomes a part of the vascular ring in the axis. The bundles are largest where they diverge from the stele, and are gradually smaller at lower levels, where they end blindly after extending through 15–20 nodes. The vascular connections within the stele are by secondary tissues and not by anastomosing of bundles. Usually 8–10 foliar bundles are differentiated below the cotyledonary node. Bundles associated with the first pair of leaves anastomose with the lateral arches of the metaxylem of the rootlike lower hypocotyl; but even in this case the bundles are small at the lower level and vascular connection is principally by secondary tissues. Other foliar bundles below the cotyledonary node end

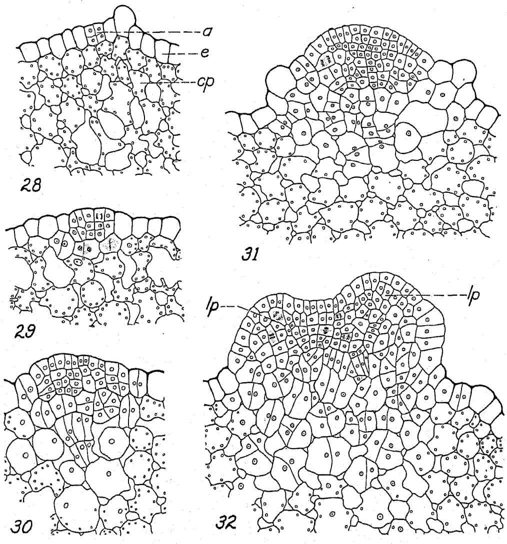
blindly in the parenchyma between other vascular bundles. As the primary tissues mature, fascicular and interfascicular cambium is differentiated. The tissues formed by the activity of fascicular and interfascicular cambium form a continuous vascular cylinder of secondary tissue, which establishes the only vascular connection between the primary vascular elements.

Adventitious buds from hypocotyl

Young plants readily produced shoots when the growing point was injured or removed. When the injury was above the cotyledon an axillary bud became active and produced a shoot. All organized buds were removed by severing seedlings of various ages at different levels below the cotyledons. All plants not more than 10 days old produced 5–20 buds on the remaining lower part of the hypocotyl, even when the top had been removed to a few millimeters above the ground level. When the tops of plants 60 days old were severed below the cotyledons, approximately 60 per cent of the plants died without forming buds; while plants less than 10 days old produced small buds along the remaining hypocotyl within 6–8 days after severing. These buds do not all start development at the same time. A microscopic examination shows that while buds on one part of the hypocotyl are large enough to show small leaves, many other buds are in earlier stages of development. After 8–10 buds begin to show small leaves, one bud usually outgrows all the others. This one bud may be at any level in relation to the others, and is not necessarily the first one to have made its appearance on the hypocotyl. In a few instances more than one bud may continue development. The shoot that develops from such an adventitious bud continues as an unbranched axis until the formation of a flower cluster, which matures at approximately the same time as those of plants of the same age left uncut. At maturity the adventitious shoot has attained about one-half the height of a plant left uncut. Many of the cells at the cut surface of the hypocotyl collapse and die, and little or no wound parenchyma is formed by the underlying cells.

After a plant has been severed, the cortical region of the remaining part of the hypocotyl develops large intercellular spaces which give it an appearance similar to the mesophyll of a leaf (figs. 28, 29).

The bud is initiated by division of an epidermal cell. This first division is quickly followed by a second, which gives a four-celled stage such as is shown by figure 28. Epidermal cells adjoining the derivatives of the first meristematic cell in turn become active, so that



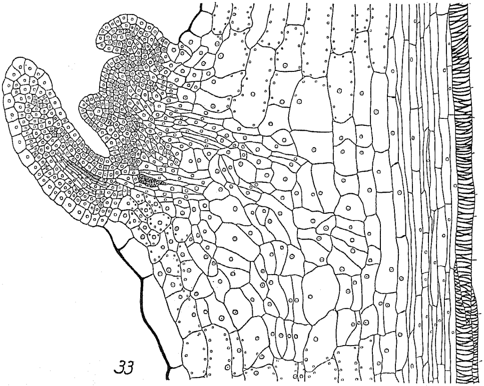
FIGS. 28–32.—Transverse sections of hypocotyl showing development of adventitious bud after plant has been severed below cotyledons: *a*, four derivatives of single epidermal cell; *e*, epidermis of hypocotyl; *cp*, cortical parenchyma of hypocotyl; *lp*, leaf primordia of adventitious bud.

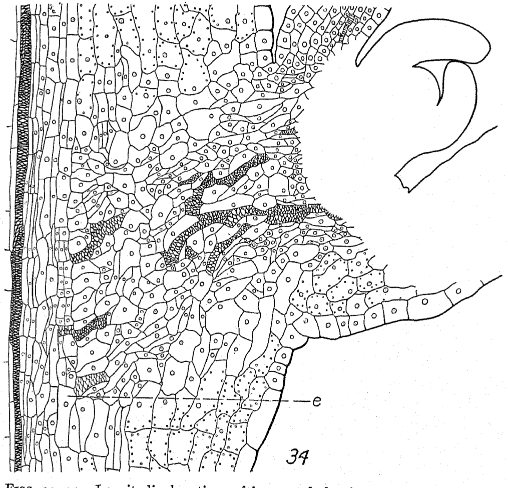
as many as five or six epidermal cells become meristematic (figs. 29, 30). The identity of the derivatives of an original epidermal cell can be determined by a heavy cell wall about the group, even after four or five subsequent divisions (fig. 30).

When one or two epidermal cells have become active, the under-

lying, adjoining cortical cells lose their chloroplasts, become less vacuolate, and begin cell divisions (fig. 29). These cells continue to divide and enlarge, so that the large intercellular spaces are filled and other adjoining cortical cells in turn become active. The activity of the cortical parenchyma continues until an active zone of cells extends to the endodermis. Growth of the cortical parenchyma results in elimination of the intercellular spaces. These new parenchymatous cells differentiate in place and do not contribute to the elongation of the new axis.

Derivatives of the original epidermal cells by subsequent divisions eventually constitute the axis of the adventitious bud. A dome-shaped group of cells is first formed (fig. 31), and then by rapid cell growth two or three leaf primordia are developed about the periphery of the dome-shaped group of meristematic cells. The first leaf primordia develop rapidly, and the flat group of cells between them later becomes a dome-shaped growing point which is similar to the epicotyl (figs. 32, 33). Vascular bundles are differentiated in the first two or three leaves of the new bud by the time the growing point is well established, and before any vascular system has been established between the stele of the hypocotyl and the new bud. Protoxylem elements are differentiated in the first leaves of the new bud before cell division has begun in the inner layers of the cortex of the hypocotyl (fig. 33). After the cells of the inner cortex have become meristematic, vascular tissue is differentiated progressively inward from the new bud to the endodermis. After many tracheids have been differentiated from the derivatives of the cortical parenchyma, a number of simultaneous tangential and radial divisions take place in the endodermis (fig. 34). Some of the derivatives of the endodermal cells soon differentiate into tracheids, and establish continuity with the tracheids which have differentiated from cells in the pericycle and in the phloem parenchyma (fig. 35). Figure 35 indicates the relative size of the adventitious shoot before vascular connection is established with the vascular elements of the primary axis of the hypocotyl. Very few cells other than elongated parenchymatous cells can be identified as phloem. By the time vascular connection is established with the vascular elements of the hypocotyl, a layer of cells surrounding the tracheids becomes an active cambium.





FIGS. 33, 34.—Longitudinal sections of hypocotyl showing development and differentiation of adventitious buds after plant has been severed below cotyledons: *e*, endodermis.

In a few weeks the secondary tissues resulting from the multiplication and divisions of the cambium of the hypocotyl and those from the cambium of the newly formed shoot develop a plant axis which is almost straight.

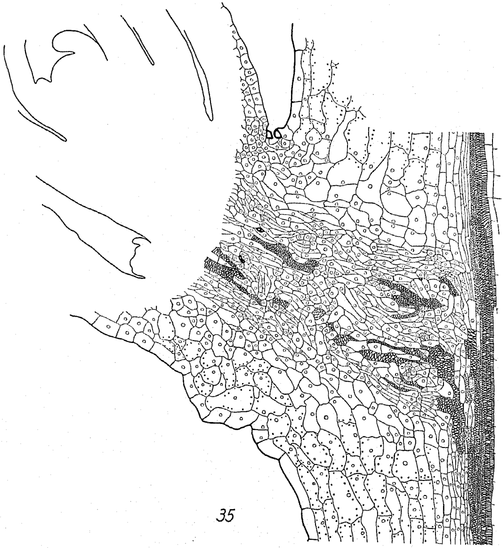


FIG. 35.—Longitudinal section through hypocotyl and an adventitious bud showing differentiation of vascular connection between the new bud and stele of hypocotyl.

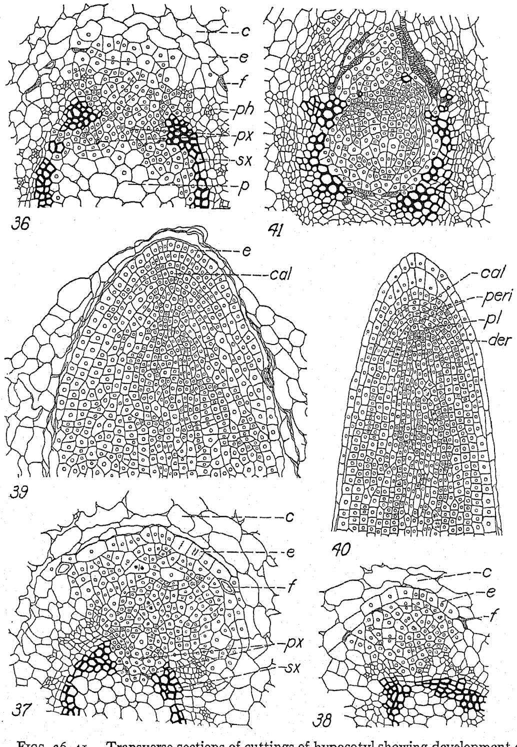
Adventitious roots from hypocotyl

Plants six to ten days old were severed at the middle region of the hypocotyl, and the upper part of the plant set with the hypocotyl in moist soil so that the cotyledons were just above the soil level. Elongation of the hypocotyl soon lifted the cotyledons from 1 to 3 cm. above the ground level, depending on the age of the

plant at the time of cutting. All the cuttings of young plants produced two to five roots along the lower part of the hypocotyl. Roots developed even when the remaining hypocotyl was only long enough to hold the cotyledons in contact. Cuttings of the epicotyl made several nodes above the cotyledons also produced roots.

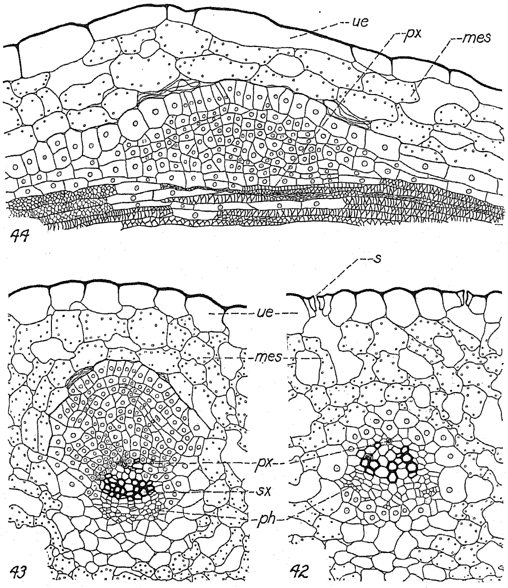
In the hypocotyl a V-shaped ray of parenchymatous tissue extends outward from each protoxylem strand, which is continuous with the protoxylem of the median bundle of a cotyledon, and the adventitious roots are usually initiated by a general activity of the cells in these parenchymatous rays. These roots then come from the hypocotyl in two rows, each of which is directly below the midvein of one of the cotyledons. Often the general activity extends from the outermost layer of the pericycle to and including the parenchymatous cells about the primary xylem (fig. 36). Almost all of the primary xylem is broken down and resorbed so that cell activity involves even the pith. Cell divisions continue in all planes, thus forming a dome-shaped group of cells which pushes outward into the cortex. The endodermis of the hypocotyl can be identified by the early development of pericyclic fibers (1). The endodermis becomes active by cell divisions in a radial plane (fig. 37). This uniseriate layer of endodermal cells keeps pace with the subsequent growth of underlying cells, and forms a layer over the root cap which does not break down until the new root grows 1–2 mm. into the soil. The new root grows by general activity of the cells, with no organized histogens until about the time it pushes through the cortex into the soil. Then at the tip of the new root the cell layer underneath the endodermal layer begins to divide periclinally, thus becoming a true calyptrogen which continues to function as the calyptrogen described for the primary and lateral roots (fig. 39). The periblem and plerome are not definitely established until the root has grown 2–3 mm. into the soil. Figure 40 shows an adventitious root with total length of 2.5 mm., in which the periblem and plerome have become established and are functioning as the histogens described for the primary root.

Cell divisions often occur throughout the pith region of the hypocotyl, and form a solid cylinder of parenchymatous cells which remain active for a month or more after the new roots develop (fig. 41). When two roots develop at the same level on the hypocotyl, the cell



FIGS. 36–41.—Transverse sections of cuttings of hypocotyl showing development of adventitious root. Figs. 36, 37, 39, initiation of roots in ray parenchyma in same radius as protoxylem. Fig. 38, initiation of root in pericycle and phloem parenchyma not in same radius as protoxylem. Fig. 40, median longitudinal section of adventitious root with histogens established (root length 2.5 mm. from hypocotyl). Fig. 41, transverse section of hypocotyl, median through an adventitious root, showing cell activity throughout pith region: *c*, cortex; *e*, endodermis; *f*, pericyclic fiber; *ph*, phloem; *px*, primary xylem; *sx*, secondary xylem; *p*, pith; *cal*, calyptrogen; *peri*, periblem; *pl*, plerome; *der*, dermatogen.

activity of the pith is usually more extensive. The cells of this region of the pith may become differentiated as wound tracheids or remain as relatively thin-walled cells.



FIGS. 42–44.—Figs. 42, 43, transverse sections of cotyledon made at right angles to median vein showing development of adventitious root: *ue*, upper epidermis; *mes*, mesophyll; *px*, primary xylem; *sx*, secondary xylem; *ph*, phloem; *s*, stoma. Fig. 42, first activity of parenchyma surrounding median vein. Fig. 43, early development of adventitious root on adaxial side of median vein of cotyledon. Fig. 44, longitudinal section through median vein of cotyledon showing origin of adventitious root on adaxial side of bundle.

Some adventitious roots of the hypocotyl are initiated by a general cell activity in the pericycle and phloem parenchyma (fig. 38).

In this case they are not in the same plane as the median bundles of the cotyledons. The subsequent development is the same as that described for the roots arising in the plane of the median bundles of the cotyledons.

At the cut surface of the hypocotyl some groups of parenchymatous cells of the cortex, pericycle, phloem, and sometimes regions of the pith divide several times without the derivative cells enlarging. Except in these regions of parenchyma, no extensive wound tissue is formed.

BEALS (3), in describing regeneration phenomena in flax, states: "First the epidermis divides and then the innermost row of those cells and the stimulated cells of the region just beneath form the regenerated part, root or shoot (pl. 16)." In the present investigation the shoots were initiated by the epidermis and the roots from tissues of the stele. Cuttings grown on filter paper in sterile dishes, in the manner described by BEALS, developed roots, all of which originated from tissues of the stele in the manner already described.

Adventitious roots from cotyledons

The cotyledons were severed from seedlings and placed on the surface of moist soil. Some were placed horizontally with the adaxial surface up, others with the adaxial surface down, and others vertically with the cut end set about 5 mm. into the soil. About 90 per cent of the cotyledons taken from plants less than ten days old developed three to six roots, while few of the cotyledons from plants twenty days old produced roots when placed on moist soil. The cotyledons developed an extensive root system and grew to be larger and thicker than those not removed from the axis. Often one to six layers of parenchymatous cells at the cut surface of the cotyledon divided several times without the derivative cells enlarging. Sometimes some of the wound tissue near the larger bundles differentiated tracheids. Cotyledons never produced buds but often lived for more than sixty days, while those on the plant usually live as active photosynthetic leaves for about thirty days.

Usually one root came from near each of the three larger veins of the cotyledon, although as many as three roots frequently developed near one vein. The roots pushed through the adaxial surface

of the cotyledon within 3–4 mm. of the cut end, or emerged from the cut surface. When a cotyledon was placed with the adaxial surface up, the roots either emerged from the cut surface or grew up through the epidermis and then curved over the cut end into the soil.

These roots were initiated by the activity of a layer of parenchymatous cells which surrounds the vascular elements of the bundle (fig. 42). In many cases this layer became active about the entire bundle, and in some cases the phloem and xylem parenchyma also became active. In other cases the activity was limited to the adaxial side next to the xylem. The cell activity usually extended 1 mm. or more along the bundle, and the cambium of the larger veins produced considerable secondary tissue (figs. 43, 44). Increased cell activity on the adaxial side of the vein produced a dome-shaped group of cells (figs. 43, 44) which was either pushed through the upper epidermis or grew about parallel to the bundle. When the roots were 2 mm. or more in length, definite histogens were organized similar to those described for the adventitious root from the hypocotyl.

In two cases the root was organized from wound tissue and grew directly from the cut surface of the cotyledon. All other roots that emerged from the cut surface, or pushed through the epidermis, had their origin in the parenchymatous cells on the adaxial side of a vein at a point 1–4 mm. from the cut surface. From the point of origin the roots either grew through the mesophyll parallel to the vein and emerged through the cut surface or pushed through the epidermis on the adaxial surface of the cotyledon. In a few cases the roots originated almost lateral to a vein but were always slightly nearer the adaxial surface.

Summary

1. Germination of the seed and development of the seedling of *Linum usitatissimum* are described.

2. The primary root is diarch. The growing point has clearly defined histogens. The plerome, periblem, and calyptrogen give rise to the stele, cortex, and root cap respectively. Laterally to the calyptrogen, the dermatogen produces the epidermis by anticlinal divisions.

3. In the development of the vascular elements of the root, two

primary phloem ducts are first differentiated by elongation and breakdown of a single row of cells lying next to the pericycle and alternate with the protoxylem points which are differentiated later.

4. Lateral roots are initiated by regular divisions of some of the cells of the pericycle. One tangential division of these cells of the pericycle forms two layers which in turn divide simultaneously and form four layers of cells. The outer layer later differentiates as the calyptrogen and dermatogen, the next as the periblem, and the innermost two as the plerome.

5. In formation of lateral roots the endodermis becomes active and forms a single layer of meristematic cells overlying the root cap.

6. A simple type of transition from root to cotyledons occurs in the hypocotyl and base of the cotyledons. The primary vascular system of the root, hypocotyl, and cotyledons forms a system largely independent of the subsequent epicotyledonary development. The principal vascular connection of the epicotyl and hypocotyl is by secondary tissues.

7. In the upper region of the hypocotyl most of the primary xylem is broken down by elongation, and is for the most part resorbed by intervening parenchymatous cells.

8. Development of the epicotyl and foliage leaves is described. All vascular bundles of the epicotyl are collateral foliar bundles which do not anastomose but end in the parenchyma between other vascular bundles. The only vascular connection between the primary vascular bundles of the stele is by a continuous vascular cylinder of secondary tissues which is formed by the activity of fascicular and interfascicular cambium.

9. The basal part of seedlings severed at the middle region of the hypocotyl regenerated five to twenty buds, of which usually only one continued development and became a shoot. Regenerated buds originated by successive division of epidermal cells. Vascular connection between the primary stele of the hypocotyl and the new shoot was initiated by renewed cell activity of cortical parenchyma, endodermis, pericycle, phloem parenchyma, and cambium, in the order named. Tracheids of the vascular system were first differentiated in the leaves of the new bud, and then differentiation occurred progressively inward to the stele of the hypocotyl until con-

tinuity was established between the tissues of the new shoot and the stele of the hypocotyl.

10. Seedlings severed in the middle region of the hypocotyl and the upper parts used as cuttings set in moist soil or placed in moist petri dishes produced adventitious roots from the hypocotyl. These roots originated from parenchymatous tissue which in the same root often involved pericycle, phloem, and pith. The endodermis of the hypocotyl became active and produced a single layer of cells overlying the tip of the developing root.

11. Cotyledons cut from seedlings and placed on moist soil produced adventitious roots and lived for more than two months. These roots originated from a layer of parenchymatous cells on the adaxial side of the larger veins. In no case did these cotyledons produce shoots.

The writer wishes to express grateful appreciation to the members of the Department of Botany of the University of Chicago for helpful suggestions and criticisms during the progress of this investigation.

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MUNCIE, INDIANA

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A NEW OCCURRENCE OF THE OLDEST KNOWN TERRESTRIAL VEGETATION, FROM BEARTOOTH BUTTE, WYOMING

ERLING DORF

(WITH PLATES V, VI)

Introduction

Although the widespread and highly organized vegetation of the upper Paleozoic era (Upper Devonian and Carboniferous periods) is profusely represented in many localities throughout the world, the primitive plants of the Lower Devonian, which are the oldest terrestrial forms thus far known, are recorded from only a few scattered localities, chiefly in western Europe, Australia, and eastern Canada. In western North America the whole of the Paleozoic system is deficient in remains of land plants, chiefly because most of the sediments are of marine origin. It is therefore of interest to report the discovery of a small but well preserved Lower Devonian flora from an estuarine deposit at Beartooth Butte, Wyoming.

The plant remains were discovered and collected during the summer of 1932 by a party of investigators including the writer, in connection with the Princeton Research Project in Montana and Wyoming. The expedition, primarily concerned with the collection of primitive fossil fishes, was conducted under the auspices of the Princeton Scott Fund, to which the writer expresses his grateful appreciation.

Occurrence

The plant impressions were found in a lens of impure limestone which outcrops on both the eastern and western cliff faces of Beartooth Butte. This butte is situated in the northern part of Park County, Wyoming, 3 miles south of the Montana line, and is an outlier of Paleozoic sediments resting on pre-Cambrian granites and gneisses. The limestone lens, which appears to be an ancient channel deposit, 175 feet thick and 2000 feet across, lies, with erosional unconformity, on the marine Bighorn dolomite of Ordovician age, and

is in turn overlain, with less marked unconformity, by the marine Jefferson limestone of the Middle Devonian. A preliminary study of the red and gray impure limestone of the lens seems to indicate deposition in a quiet, shallow, drowned valley along a submerging coast line. Absence of peaty layers suggests that swampy conditions did not prevail, and that the plants grew as a fringe around or possibly in the shallow borders of the basin.

In direct association with the plant remains occur numerous well preserved fish plates. A preliminary report (4) on the fish fauna collected during the summer of 1931 indicated an abundance of Ostracoderms and Arthrodires. The assemblage is clearly of Lower Devonian aspect.

The plant fossils were collected partly *in situ* and partly from large talus blocks on the steep slopes below the outcrop. Unfortunately no petrifactions were discovered, so that very little is as yet known regarding the detailed internal structure of the plants. Attempts to study the microscopic details of the impressions by the film-transfer or collodion film-pull methods, or by maceration in acids, have thus far been unsuccessful, owing to the fact that very little carbonaceous material has been left adhering to the rock matrix.

Description of species

The descriptions are based entirely on the megascopic details. It is likely that if better material is procured from this locality, microscopic study will warrant more definite determinations. However, five distinct types are described and discussed in detail:

1. *Psilophyton wyomingense*, new species: flattened spiny branch systems.
2. (*?*) *Psilophyton* sp.: an elongated fructification (?).
3. *Bucheria ovata*, new genus and new species: narrow axes with clustered, terminal sporangia (?).
4. *Hostimella* sp.: flattened branch systems without spines.
5. (*?*) *Bröggeria strobiliformis*, new species: cylindrical fructification of strobilus-like nature.

All of these forms are referred to the primitive order Psilophytales. The generic determinations of types 2 and 5 are unfortunately tentative and somewhat questionable, because of scarcity of material in

the collections. The remaining three types are each represented by twelve or more well preserved specimens.

PSILOPHYTON Dawson

Psilophyton wyomingense n. sp. (figs. 2–6, 8)

DESCRIPTION.—Somewhat flexuose branch system with main axis and subequally dichotomous lateral branches, probably sympodially formed. Lateral branches given off from main axis at acute angles and repeatedly bifurcating dichotomously at similar low angles, apparently in one plane. Surfaces irregularly and obscurely marked with rounded aréoles, presumably spine scars, and with occasional fine longitudinal striations. Entire branch system bearing closely set spines which are irregularly disposed and more numerous on main axis. As seen in profile, spines are of various lengths up to 2 mm. and are extremely fine, tapering from an only slightly enlarged basal portion to a pointed distal end.

DISCUSSION.—This species is represented in the collections by numerous flattened, spinous branch systems whose characteristics are so closely comparable with those of previously described and figured species of *Psilophyton* that its reference to that genus is unquestionable. It is indeed difficult to differentiate this form from the original species, *P. princeps* Dawson, even in its narrowest sense (=var. *ornatum*) as redefined by HALLE (10) and WHITE (30).

A comparison with DAWSON's original figures of the typical spine bearing branches of *Psilophyton princeps* (7) brings out the following differences which seem to necessitate defining the Wyoming form as a separate and distinct species: (1) lateral branches are more repeatedly bifurcated, unlike those of *P. princeps;* (2) branch tips do not show the characteristic circinate habit of vernation; (3) spines are finer, more closely spaced, and do not enlarge to so wide a base as in DAWSON's specimens. The same general similarities and specific differences are apparent in comparisons with other figured specimens of the spiny branches of *P. princeps* from France (BERTRAND 2), Scotland (LANG 20), and Norway (HALLE 10). The long fine spines of our species agree more closely with those of *P. goldschmidtii* Halle from Norway (10). In this latter species, however, the main axis is considerably larger than the lateral branches, which bifurcate at

wider angles; also the spines are much less numerous on the thicker branches and virtually absent in the thin laterals. These differences, which are somewhat less conspicuous in comparison with the German forms referred to this species (15), would seem sufficient to warrant a new specific determination for the Wyoming form.

A striking similarity to the Wyoming type is seen in the single species recorded from the archipelago of Bulandet in western Norway (26). Unfortunately this form was designated only as *Psilophyton* sp., although HALLE (10) regards it as a typical representative of *P. princeps*. More recently KRÄUSEL and WEYLAND (13) have intimated that it may well be a thin branch of *Asteroxylon elberfeldense*.

At Beartooth Butte, in the absence of more complete specimens of the entire plant, there remains the possibility that the forms here referred to *Psilophyton wyomingense* may actually be the outer branches of a larger plant whose basal portions have not yet been found. Spiny stems, formerly referred to *P. princeps*, have recently been found in direct connection with larger stems of the *Thursophyton* type in Middle Devonian beds in Germany (13). These are referred to the species *Asteroxylon elberfeldense*, whose morphological characters are now almost completely known. Until entire structures are found, however, it seems best to make no assumptions regarding the basal portions of our species and to retain the generic name *Psilophyton*.

In this connection it is possible that the specimen shown in figures 4, 5, and 8 may represent the larger basal branches of our species. The figures show the spiny branches arising almost perpendicularly from one side of a somewhat larger spiny stem (rhizome?). The fine, pointed spines, the numerous spine scars, the low-angled dichotomous branching, and the faint longitudinal striations are all characters possessed in common with the type specimen just described. The writer therefore feels justified in suggesting that this specimen can be referred to *Psilophyton wyomingense* in spite of the absence of an actual connection with the type of the species. A possible basal portion with such truly *Psilophyton*-like characteristics suggests that the *Thursophyton* type of larger branches did not exist in the Wyoming flora.

It is unfortunate that little is as yet known regarding the finer branches and possible fructifications of this species. The thin

axis shown in figure 6 possesses essentially the same characters as the lateral branches of the type specimen, being only somewhat thinner and less spinous. The more widely spaced and less numerous spines agree with other figured specimens of the outer branches of *Psilophyton* (HALLE 10, LANG 20). It is possible that some of the very thin dichotomous branches in our collection which are entirely lacking in spines may represent the extreme ends of the spiny types. The fructifications, still unknown in connection with spiny axes, may possibly be the form to be described later as (?) *Psilophyton* sp., which is similar in size and shape to the fructifications of *Psilophyton* described from elsewhere. For that matter it is not at all certain that the narrow spineless axes bearing clustered fertile organs, described here as *Bucheria ovata*, may not have been the outer branches of the spiny stems of *Psilophyton wyomingense*.

In summation, the Wyoming specimens are clearly comparable with the typical branch systems found elsewhere in Devonian rocks and referred to the genus *Psilophyton*. While described as a new species whose entire growth form is at present incompletely known, a close similarity is suggested with the more authentic forms of *P. princeps* and *P. goldschmidtii*. In the most recent contribution to the study of Lower Devonian floras, LANG (20) emphasizes the fact that in the re-examination of the floras of the Lower Old Red of Scotland it is impossible to make a clear specific distinction between specimens of the *P. princeps* type and those of the *P. goldschmidtii* type. He prefers to consider the latter as a form of the more comprehensive species *P. princeps*.

(?) **Psilophyton** sp. (fig. 1)

Only a single specimen of this type has been found at Beartooth Butte. It is doubtfully referred to *Psilophyton* until such time as material may be found to enable a more accurate determination. As seen in figure 1, the specimen is an elongate, linear, pointed body which resembles in size and shape the spore cases borne individually on the terminal ends of branches of the *Psilophyton* type. Spore cases from eastern Canada figured by DAWSON (7) and by LANG (19) are of essentially the same character as the single specimen here shown. Other closely comparable forms are those from the Lower

Devonian of Matringhem, northern France (2), and those referred to *Dawsonites arcuatus* Halle from Norway (10).

Bucheria, n. gen.

DESCRIPTION.—Spineless, narrow axes bearing at their distal ends clusters of small rounded appendages, which are bilaterally-symmetrically arranged on opposite sides of the axes. Appendages closely set, sessile, and opposite to subopposite.

Bucheria ovata, n. sp. (figs. 9–17)

DESCRIPTION.—Long, narrow axis bearing at distal end, in two rows, clusters of small ovate bodies, symmetrically arranged on opposite sides of axis. Axis averaging 1.8 mm. in width and up to 6.5 cm. in length, non-spinous, and marked by a few pronounced longitudinal costae and numerous faint interrupted striations. Lateral bodies up to sixteen in number, closely set, sessile, and somewhat pointed at their distal portions. Dorsal and lateral views of these bodies often showing longitudinal median splits extending from their central areas to their pointed ends. Microscopic structural details unknown.

DISCUSSION.—This species, which is represented in the collections by twelve or more well preserved specimens, is at present restricted to what are undoubtedly the fertile portions of a plant whose vegetative parts are as yet unknown. Although proof is still lacking, it is reasonable to suppose that the impressions are of leafless axes bearing terminal spikes of sporangia. Innumerable attempts to study the microscopic details by the film-pull or film-transfer methods have been unsuccessful, so that their sporangial nature is still an assumption based on their general appearance and on comparisons with European and Australian forms in which spores have actually been found (22).

As seen in figures 14–16, there is in several of the bodies a clear distinction between an outer rim and an inner, solid structure. The solidity and distinctness of this central area are due to sediment which apparently filled in a hollow cavity at the time of preservation. That it had previously contained spores is suggested as a possibility. The outer layer may be a thickened epidermis. In a recent

letter from Dr. LANG, he states that film-pulls made on two specimens sent to him "have yielded appearances suggestive of a distinction of wall and spores, but only suggestive."

A somewhat more convincing detail is the definite split clearly seen in the dorsal and lateral views of several specimens (figs. 9–12, 15, 16). In a few of the forms, particularly figure 15, this split is seen to continue to the distal end where it divides the structure into two bluntly pointed lobes. This type of split may well have been the line of dehiscence in the individual sporangia, in which case the inclosed spores had undoubtedly already been liberated before preservation.

The absence of radial symmetry in this species suggests a dorsiventral arrangement of the sporangia on their axes. Since dorsiventrality is normally found only in plagiotropous (oblique or horizontal) portions of plants, it seems plausible to regard these impressions as fertile leaves, or sporophylls, rather than as central spikes, in which radial symmetry would be present. This observation intimates that the axes are rachises of divided fronds rather than of stem nature.

In the comparison of *Bucheria ovata* with other Devonian fructifications, none has been seen which possesses characters similar enough to warrant specific, or even generic, identity. One form referred to as ? *Dawsonites* sp., from the Lower Devonian of Willwerath, Germany (16), might possibly belong to the same genus. The figured specimen (16, text-fig. 52) shows a narrow axis terminated by two definite rows of oval, elongated organs (spore cases ?) and a possible third row indicated by one of the bodies assuming a central position between the lateral rows. It is reasonable to suppose from the slight displacement of several of the bodies that this single erratic one might well have been shifted out of its normal position in the process of preservation. If that be true, as seems likely, it would be better to place both the Wyoming and the German forms in the new genus *Bucheria.* The genus *Dawsonites*, to which the German specimen is questionably referred, is defined by HALLE (10) as having dichotomously dividing curved branches bearing single terminal spore cases which are non-sessile and narrowly obovoid or short fusiform.

None of these features is evident in *Bucheria ovata* or the German ? *Dawsonites* sp.

In addition to ? *Dawsonites* sp., the Wyoming form also presents a fair comparison with the "cones" of *Arthrostigma gracile* from the Lower Devonian of Campbellton, Nova Scotia (8, pl. XXIV). As figured, this specimen seems to have been incompletely and poorly preserved, showing only the two rows of crescent-shaped bodies without indicating their connection with an axis or their morphological details. DAWSON (8) intimates that they may have been "spikes of sac-like spore cases."

From the Lower Old Red of Scotland, *Zosterophyllum myretonianum* Penh. shows a somewhat similar morphological construction (LANG 18). This species has clustered, terminal spore cases differing from the Wyoming types mainly in their reniform shape, radial arrangement, and wider spacing on their axes. *Z. australianum* L. & C. (22) from the Lower Devonian of Australia is equally similar in general composition. The resemblance of our form to both these species of *Zosterophyllum*, in which actual spores within the appendages have been definitely demonstrated (22), is particularly significant in intimating the sporangial nature of the appendages in *Bucheria ovata*. Another species based on clustered, terminal sporangia is *Hostimella racemosa* Lang from the Middle Old Red of Scotland (17). While this also is comparable with *Bucheria ovata*, it differs considerably in that its spore cases are stalked, more pointed, and much more widely spaced on the axes.

In the absence of a definite connection between the terminal clusters of sporangia here referred to as *Bucheria ovata*, and larger branches or stems, it is impossible to ascertain just what the vegetative portions of this plant looked like. The spineless nature of the long narrow axes bearing the fructifications suggests a connection with the naked stems described in a following paragraph as *Hostimella* sp., although there remains the possibility that they may belong to *Psilophyton wyomingense*, whose end branches might have been spineless. That this view is not a far-fetched probability is shown by the discovery of naked spore-bearing branches at the ends of spiny stems of the *Psilophyton* type in *Asteroxylon elberfeldense* from

the Middle Devonian of Germany (13). On the other hand, the presence in the Beartooth flora of a sporangium (?) of the *Psilophyton* type (discussed here as ? *Psilophyton* sp.) makes it more plausible to assume that this type of single fructification was borne by the spiny branches of *P. wyomingense*, and that the clustered spore cases (*Bucheria ovata*) were developed on branches of another type, perhaps *Hostimella* sp.

The preceding description and discussion leave much to be learned as to the true nature of these clustered fructifications. Despite the lack of more complete material, the many well preserved and well defined specimens in the collections seem to justify the new generic name *Bucheria*. This designation is intentionally noncommittal as to systematic affinities, which are at present doubtfully known. It is with pleasure that the writer has named the genus in honor of Dr. and Mrs. WALTER H. BUCHER, of Cincinnati, Ohio, who were members of the 1932 expedition to Wyoming.

HOSTIMELLA P. & B.

Hostimella sp. (figs. 18, 19)

The collections from Beartooth Butte contain about twelve thin, dichotomously branched stems without spines. Similar forms are recorded from most of the Devonian localities elsewhere and are usually referred to the form type, *Hostimella* sp., although there is no very definite line of demarcation between this designation and *Aphyllopteris* (22). Included as *Hostimella* sp., however, are two types of smooth axes differing chiefly in the presence or absence of peculiar axillary "budlike" structures. These are to be seen in several specimens from the Middle Old Red of Scotland (17), the Walhalla series of Australia (22), the Middle Devonian of Germany (12), Belgium (5), and Bohemia (27). In none of the Wyoming specimens is there any trace of an "axillary bud." With the exception of such forms as do possess these peculiar structures, it is difficult to distinguish the Wyoming specimens from most of the figured specimens of *Hostimella* sp., from either the Lower or Middle Devonian. Those recorded by HALLE (10) from the Lower Devonian of Norway are perhaps the most strikingly similar.

Only recently has a little light been cast on the systematic position of these common Devonian forms. Naked, dichotomous branches, formerly referred to *Hostimella hostimensis* P. & B., have been found in direct connection with spiny stems of the *Psilophyton* type in Germany; the complete plant is now known as *Asteroxylon elberfeldense* Kr. & W. (13). This does not mean, of course, that all such branches found elsewhere must necessarily have been connected with spiny stems, but at least it presents that possibility as more than an unfounded assumption. In the Wyoming forms there is some reason to believe that this was not the case; a few of the naked branches are fully as thick as those of *Psilophyton* bearing many well defined spines; also the thinner branches without spines are not smaller than those of *Psilophyton* which do bear spines.

It is obvious that these naked branches must remain as of unsatisfactory classification, despite their common occurrence in Lower and Middle Devonian floras, until such time as they may be found in direct association with larger stems of similar or different characteristics. In the present flora no such complete specimens have been found.

That the terminal ends of the naked branches of *Hostimella* sp. might have borne the fructifications described as *Bucheria ovata* has been mentioned in the discussion of the latter species.

Bröggeria Nathorst

(?) **Bröggeria strobiliformis** n. sp. (fig. 7).

This species is represented in the Wyoming collections by only a single specimen. In this, however, the characters are well defined and of a decidedly new type, worthy of recognition as a matter of record.

As shown in figure 7, the species is based on an impression of a cylindrical, strobilus-like body studded with numerous small, rounded bodies. It resembles in general appearance the terminal, sporangia-bearing catkins of Equisetaceous types. Until much more material of this type may be discovered and studied, the determination must remain as unsatisfactory and questionable.

The tentative reference to *Bröggeria* is based on comparisons with

a species recorded from the Lower Middle Devonian of western Norway (26), but the similarity is none too great. The writer feels certain that the Wyoming form cannot be definitely referred to any previously recorded genus. It would seem unreasonable, however, to describe a new genus on such insufficient data as our single specimen affords.

Bröggeria norvegica Nat., as figured on plates 3 and 4 (26), is clearly of strobiloid nature. It is considerably larger than the Wyoming type and exhibits a much more open and loose arrangement of sporangia (?). It also possesses short, leaflike appendages which separate the individual bodies from one another. This feature is not evident in our specimen. In general appearance, however, the Norwegian species is the only Devonian form with which a comparison is at all possible.

Correlation

In attempting to determine the exact age of the plant-bearing beds at Beartooth Butte, several significant factors must be kept in mind: (1) Comparisons must of necessity be with floras which are far distant from the Beartooth flora; such long-distance correlation is not entirely satisfactory. (2) The Beartooth flora, consisting of only five distinct forms, is too small for accurate comparison with larger floras elsewhere. (3) None of the species is identical with any recorded from other localities, so that conclusions must be drawn from index types rather than from index species. (4) The exact ages of the Canadian and European Devonian floras, with which comparisons are made, are by no means definitely settled. Obviously, unless the stratigraphic position of such floras is determined by superposition and associated animal remains, the exact age references cannot be made until the Devonian floras are better known.

Fortunately the age determination of the Beartooth beds is helped by the stratigraphic relations. Unconformably below the beds lies the Bighorn dolomite of Upper Ordovician age (MILLER 25); above lies the Jefferson limestone of the Middle Devonian (LOVERING 24). We are thus clearly dealing with a formation of either Silurian or Devonian age. The possibility of Silurian age is extremely remote; no authentic terrestrial floras have thus far been recorded from Silurian rocks anywhere in the world. The fish fauna which occurs

in direct association with the plant remains is distinctly of Lower Devonian aspect (BRYANT 4). Evidence will be presented to indicate that the plant remains substantiate the age reference based on the fish remains.

Considering the Beartooth flora as a whole, it is apparent that we are dealing with a representative of the so-called *Psilophyton* flora (ARBER 1) of the early Devonian. This type is replaced in the upper part of the Middle Devonian by the well known and more highly organized *Archeopteris* flora (13). It is thus relatively certain that the Beartooth flora, aside from stratigraphic and other paleontologic implications, must be older than the Upper Middle Devonian.

Among the European representatives of the *Psilophyton* flora, the association of *Psilophyton goldschmidtii*, *Hostimella*, and ? *Dawsonites* sp. from the Lower Devonian of Willwerath, Germany (KRÄUSEL and WEYLAND 16) is of essentially the same character as the Beartooth flora. The three Willwerath types are among the most similar correlatives of the three most abundant Beartooth species, *Psilophyton wyomingense*, *Hostimella* sp., and *Bucheria ovata*, as previously stated. Other floras which are comparable with the Beartooth flora as an association of types are the Campbellton of eastern Canada (7), the Lower Old Red of Scotland (20), and the Röragen of Norway (10), all of which are considered of Lower Devonian age. While these observations tend to substantiate the evidence of the associated fish remains and the stratigraphic position for a Lower Devonian age reference, it may be instructive to compare the individual forms in the Beartooth flora with related forms elsewhere.

Psilophyton wyomingense is well represented in the Beartooth collections. As already pointed out, its nearest relatives, as judged by close similarity in megascopic characters, are *P. princeps* and *P. goldschmidtii* (both of widespread distribution), and *Psilophyton* sp., from Bulandet, Norway. The typical spine-bearing stems of *Psilophyton* are especially characteristic of the Lower Devonian (10), as exemplified by their occurrence at Campbellton (7), Matringhem (2), Röragen (10), and in Scotland (20). LANG (17) reports that authentic specimens of *P. princeps* are known only from the Lower Old Red of Lower Devonian age, although the "name has frequently been loosely applied to remains" in the Middle Old Red of Lower to Mid-

dle Devonian age. *P. goldschmidtii* is confined to the Lower Devonian and is considered to be one of the most widespread Lower Devonian species of Europe (15). The third correlative, *Psilophyton* sp., from Bulandet, Norway, is likewise referred to the Lower Devonian (10).

Bucheria ovata, which is also abundant in the Beartooth flora, is of the same general construction as ? *Dawsonites* sp., the "cones" of *Arthrostigma* and *Zosterophyllum*. The described and figured specimen of ? *Dawsonites* sp. is of a type which is recorded from only one European locality; the beds in which it occurs are referred to the Lower Devonian (16). In eastern Canada, the "cones" of *Arthrostigma* are likewise confined to the Lower Devonian (8). The same age determination is given for the beds containing *Zosterophyllum myretonianum* (18) and *Z. australianum* (22).

Hostimella sp. possesses less distinctive characteristics, which detracts from its usefulness as a guide fossil. In all of the Beartooth specimens, however, there is a notable lack of the small "axillary buds" observed in *Hostimella* sp. from several European localities. LANG and COOKSON (22) have pertinently pointed out that these axillary budlike structures are not known in the *Hostimella* branches of the Lower Devonian in Europe but are recorded from several scattered horizons in the Middle Devonian. Since this publication, "axillary buds" have been found in the Lower Devonian of Scotland (20), but are not abundant and cannot be considered as characteristic. While such negative evidence is of doubtful value when considered alone, it does afford additional corroboration of the Lower Devonian age reference indicated by *Psilophyton wyomingense* and *Bucheria ovata*.

The two forms (?) *Bröggeria strobiliformis* and (?) *Psilophyton* sp. are represented in the collections by only one specimen each, neither of which is considered distinctive enough to render a definite generic determination. They are consequently of little or no value as indices of geologic age.

From the foregoing discussion it seems apparent that the combined evidence derived from the plant remains, the associated fish remains, and the geologic relations of the fossiliferous beds is entirely indicative of a Lower Devonian age reference.

Botanical considerations

Perhaps the most significant result of the Beartooth Butte discoveries is the extension into western North America of the early Devonian *Psilophyton* flora, which was apparently more widespread and uniform than might have been suspected.

It is also becoming more evident that the spiny branch system of *Psilophyton princeps* and its close relatives were among the most widespread of the Lower Devonian forms in the northern hemisphere, and that the clustered spore cases represented by *Bucheria ovata* were by no means as limited as is indicated in the European record.

The chief botanical interest in the Beartooth flora unquestionably lies in the questions which it raises regarding the true nature of these simple, ancient plants. That they were truly terrestrial is at present merely a deduction based on their similarities to European forms in which vascular tissue, cuticularized epidermis, and stomata have been shown to be present by microscopic studies. It is possible that the faint, longitudinal striations on many of the Beartooth branches may be due to the elongated cells of a hypodermal layer, but as yet no positive evidence of structure of any kind has been obtained.

While discussions continue as to the true systematic affinities of these primitive Psilophytales, it is impossible to trace the ancestry of the more highly organized phyla of later eras. Although the Psilophytales are generally considered to be the simplest and most primitive of the pteridophytes, Arber (1) regarded them as highly advanced terrestrial thallophytes, and Church (6) placed *Hornea*, a typical Psilophytalean, in the bryophytes. Nor can we be certain, as Scott (28) has pointed out, that their simplicity was wholly primitive and ancestral; perhaps unfavorable conditions of growth had already at this early date produced a certain amount of reduction. While such a possibility may apply to the fossiliferous peat deposits of Scotland, there is no geologic evidence to intimate that the Beartooth flora grew in an abnormal or unfavorable habitat. That the spines of *Psilophyton*, moreover, were emergences and not reduced leaves has been suggested by their irregular disposition and the diversity in size in any given area (10); more recent microscopic investigations (9, 19) have substantiated such suggestions and have indicated that the spines were probably of secretory glandular nature.

There seems to be a growing conviction, corroborated by increasing evidence, that the Microphyllae and Macrophyllae, and probably also the Articulatae, can be traced back to the Psilophytales (11). In the Beartooth flora only microphyllous forms are found, unless the dorsiventral symmetry of the axes of *Bucheria ovata* and *Hostimella* sp., and the blunt tapering (intimating a limited growth) of such axes might be regarded as suggestive of megaphyllous rachises (10). It is possible that the dorsiventral sporophylls (*Bucheria ovata*) may be of the type which in the course of descent could have become sterile and have developed into broader foliage leaves.

The problem of the relation of radial to dorsiventral symmetry is by no means elucidated by the Beartooth discoveries. BOWER (3) believes that radial symmetry in the sporophyte is primitive and dorsiventrality a secondary condition. LIGNIER (23) and TANSLEY (29) adopt the opposite view. In *Bucheria ovata* the symmetry is distinctly dorsiventral; similar types of fructifications from other horizons of the same age present a definite radial symmetry, however, leaving the question of the ancestral type unsettled. In the dorsiventrality of *B. ovata*, however, there is a possibility of relationship to the primitive Filicales, in which dorsiventrality is the normal symmetry, as contrasted with the usual radial symmetry of the Lycopodiales, Sphenophyllales, and Equisetales.

It is interesting to note that a comparison of the clustered fructifications of *Bucheria ovata* with herbarium material at the New York Botanical Garden brought out a general similarity in morphological construction to several of the simple ferns, such as *Botrychium* and *Ophioglossum*. While the writer feels that the Lower Devonian floras are far too distantly removed in time from our present flora to attempt to establish systematic affinities, the comparison at least indicates the presence in the Lower Devonian of a type of fructification which is not radically different from the arrangement in several simple, modern ferns.

With respect to the higher groups of plants there is in (?) *Bröggeria strobiliformis* a suggestion that the Equisetales are represented in the Beartooth flora; this species is based on the small, strobiloid catkin whose appearance is essentially as in the Equisetales. More and

better material will have to be found, however, before this suggestion, as well as others, can be interpreted as a definite indication of relationship.

For helpful suggestions and criticism the writer is indebted to Dr. DAVID WHITE and Mr. CHARLES B. READ of the U.S. National Museum, to Professor R. KRÄUSEL of the University of Frankfort, Dr. T. G. HALLE of Stockholm, Professor A. C. Seward of Cambridge; and particularly Professor W. H. LANG of the University of Manchester, who, in addition to making many valuable suggestions, kindly examined several specimens in search of microscopic details.

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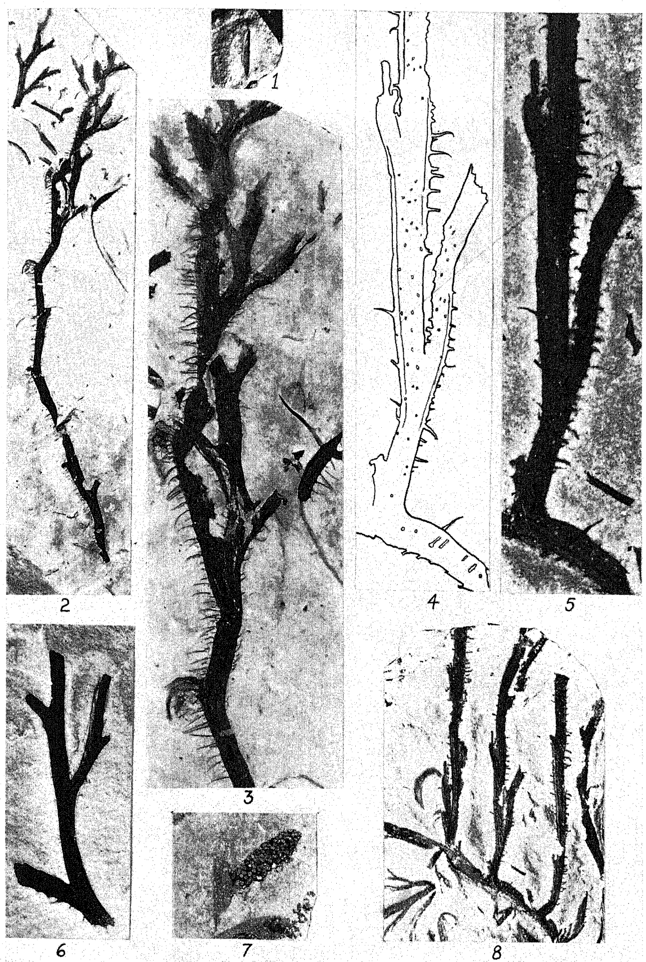
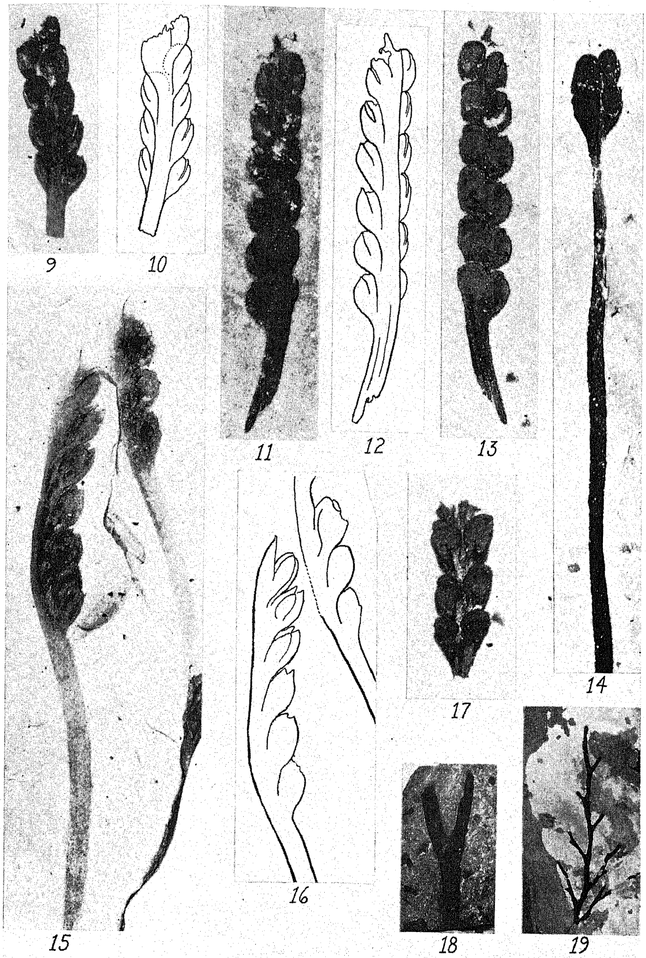


DORF on EARLY DEVONIAN



DORF on EARLY DEVONIAN

EXPLANATION OF PLATES V, VI

PLATE V

FIG. 1.—(?) *Psilophyton* sp., a single detached spore case (?); × 0.833.

FIGS. 2, 3.—*Psilophyton wyomingense*, type specimen, showing mode of branching and numerous spines. Fig. 2, × 0.833; fig. 3, × 2.5.

FIGS. 4, 5, 8.—*P. wyomingense*, showing typical spiny branches with faint longitudinal striae and prominent scars; upright position of branches suggests that the oblique branch may be a rhizome. Figs. 4, 5, × 2.5; fig. 8, × 0.833.

FIG. 6.—*P. wyomingense*, showing only few small spines on otherwise naked, outer branch. × 4.58. (Photograph by W. H. LANG.)

FIG. 7.—(?) *Bröggeria strobiliformis*, showing small rounded spore cases (?) in a catkin. × 2.5.

PLATE VI

FIGS. 9–17.—*Bucheria ovata*, fructification consisting of ovate spore cases (?) in terminal cluster; figs. 9, 10, dorsal views showing medial splits; figs. 11–13, type specimen and its counterpart showing both dorsal and ventral impressions; fig. 14, broken cluster showing long spineless axis; figs. 15, 16, lateral views of two incomplete clusters showing distal splits and prominent central area; fig. 17, ventral view. × 2.5.

FIGS. 18, 19.—*Hostimella* sp., showing dichotomous branches without spines. × 0.833.

SOME PHYSICO-CHEMICAL PROPERTIES OF SEED EXTRACTS[1]

CONTRIBUTIONS FROM THE HULL BOTANICAL LABORATORY 447

CHARLES A. SHULL AND JOHN W. MITCHELL

(WITH ONE FIGURE)

Introduction

Following the processes of fertilization of the egg and polar nuclei in the embryo sac, the development of the embryo and its associated reserve food tissues is marked by the rapid accumulation and condensation of the food materials translocated to the ovule. Soluble salts either come from the soil solution directly, or they are withdrawn from other regions of the plant where these salts have been temporarily in use or from regions undergoing senescent degradation. Soluble organic constituents come from the regions of manufacture, or from tissues in which temporary storage has taken place in anticipation of the needs of the embryo. These substances furnish the building material for the permanent cellular structures of the embryo itself, and for the deposition of its food supplies either within its own cells or in adjoining or surrounding tissues. Because the physiological processes involved during development of the seed are predominantly condensations, the embryo and its food reserves are made up very largely of relatively insoluble compounds, polysaccharide carbohydrates, oils and fats, proteins, protoplasm, and salts of these organic substances or their chemical relatives.

The translocation forms of all of these substances are soluble compounds. As these soluble constituents reach the centers of use, and are converted into insoluble tissue components and food reserves, the osmotic concentration of the cell sap of the embryonic and endosperm cells is kept low. Indeed it is possible that this maintenance of low osmotic concentrations in the region of building and storage is an important means by which the embryo makes its demands upon

[1] This investigation was aided in part by a grant to the University of Chicago from the Rockefeller Foundation.

the remainder of the plant body for its necessary supplies of building materials. For, as MASON and MASKELL (3) have shown, translocation usually occurs in a direction correlated with general osmotic gradients. This, however, should not prevent us from recognizing the possibility, or even probability, that the plant can, and perhaps often does, move its soluble materials in directions contrary to osmotic gradients.[2] Even when condensation does not go far enough to produce actual insolubility, as in the formation of cane sugar, glucosides, inulin, polypeptids, etc., one of the great advantages derived by the cell from its condensation activities is maintenance of reasonable levels of osmotic concentration within its body.

In the drying seed, of course, osmotic activity of the soluble materials is limited by the deficiency of water. Since osmotic pressure is really the pressure of water flowing into a region because of a difference in the forces which govern water balance, there seems to be little possibility of the development of strong pressures in the cells of embryo and endosperm tissues during the later stages of development of these structures; for there is too little water present to permit the soluble materials to create the typical unbalanced water pressure. The forces of movement may be unbalanced, but if the water is held tightly in the colloidal materials, it may not flow rapidly, just as in soils the film water movement becomes negligible when it reaches a certain thinness of film.

It is a well known fact that air dry substances of seeds have a very powerful attraction for water. The attraction between the water and solids of the seeds is mutual, and in part the result of molecular and ionic surface forces. This mutual attraction when measured is found to be at least hundreds of atmospheres. And oven dried seeds and water attract each other with forces of thousands of atmospheres. The causes of the attraction are never simple, but involve colloidal forces, surface chemistry, hydration, capillarity, etc., all of which may be summed up as imbibition forces. Only a small amount of the attraction is caused by the presence of soluble substances deposited

[2] While this paper was in press, a paper was published by E. PHILLIS and T. G. MASON on the polar distribution of sugar in the foliage leaf, in which movement of sucrose against its gradient of diffusion from mesophyll cells to phloem of the fine veins is apparently demonstrated (Memoir no. 4, Cotton Research Station. Trinidad. 1933).

in the air dry cells of the embryo and endosperm. It must not be forgotten, however, that if any important quantity of soluble compounds were to be held in solution in the small amount of water held by the air dry seed substance, the osmotic concentration might be fairly high, even when the osmotic pressure is nil.

Some years ago attention was drawn to the water-soluble substances of air dry seeds in a way that challenged the interest of the senior writer. BOUYOUCOS and McCOOL (1), who were interested in the "bound" water which organic matter holds against the crystallizing forces of freezing, found that from one-fourth to three-fourths of the water imbibed by seeds could be held in unfrozen condition in the presence of freezing temperatures. Such protein-high seeds as alfalfa would imbibe as much as 127 per cent of their own weight of water, and almost 75 per cent of this imbibed water remained unfrozen at -3° C.

In order to determine the quantity of soluble material in the seeds, they ground them to a fine powder, treated definite quantities of the seed powder with definite quantities of water, and after a definite lapse of time determined the freezing point depression of the mixture by means of the Beckmann apparatus. Thus 10 gm. of powdered spring wheat, plus 20 cc. of water, after standing 40 minutes for solution of material, gave a freezing point depression of -0.280° C.; alfalfa seed powder gave a depression of -0.610° C.; and speckled wax beans a depression of -1.180° C. These depressions indicated osmotic concentrations (with this degree of dilution) of 3.375 atm. in the spring wheat powder, of 7.349 atm. in alfalfa, and of 13.336 atm. in speckled wax beans. As these depressions were obtained after adding relatively large volumes of water to the dry materials, these investigators argue for very high osmotic concentrations of soluble materials in dry seeds. They attribute to this great concentration of readily soluble materials in the cells of dry seeds their enormous power to abstract water from even relatively dry soils.

Without attempting any quantitative studies of their extracts, BOUYOUCOS and McCOOL offered a suggestion as to the nature of the soluble materials which might be responsible for the high osmotic concentrations indicated by their freezing point determinations. It was this suggestion that challenged the interest of the senior writer

many years before an opportunity presented itself to test their conclusions. In the first place, they assumed that there are no soluble sugars in dry seeds, since the condensation processes lay them all down as starch, cellulose, and other insoluble hexosan and pentosan residues. It was argued, also, that there are not sufficient free salts in seeds to account for the observed depression of the freezing point in seed powders, and that therefore the soluble proteins, such as seed albumens, are mainly responsible for the highly concentrated solutions. To quote their concluding statement: "All evidences, therefore, point to the proteins as the main class of constituents in seeds which produced such high depressions in the freezing point when dry seeds in the powdered form were mixed with water."

One cannot take this statement literally, of course, as there was really no evidence submitted with regard to any of the constituents, only arguments based on common beliefs in regard to sugars and salts. In view of the high molecular weight of the simplest of soluble proteins, it would seem necessary to "dissolve" something like 25 gm. of protein in 1 gm. of water to produce a freezing point depression of $-1.18°$ C.

In this paper the writers present some evidence obtained over a period of years, the results of studies of seed extracts with special reference to the quantities of salts, sugars, and proteins which go into solution from powdered seeds. When the work was begun, some years ago, it was not realized that the exact nature of a seed extract would be so largely influenced by the manner in which it is made. It was believed that very cold (near freezing) temperatures would prevent serious autolytic changes in the carbohydrates and proteins. Pure solutions of sugars hold up remarkably well for long periods of time at temperatures of 33°–34° F., as will be mentioned later. But it became evident, as the work proceeded, that unless precautions are taken to prevent autolysis there is enough to change the quantities of soluble materials in the extracts. Our results, based upon extracts made at different times and places, and with unperceived differences in technique, are not always in such close agreement as one would wish. But they are recorded as a contribution to our knowledge of seed extracts and their physico-chemical behavior. The first studies were begun at West Virginia University in 1928.

The senior writer is greatly indebted to Dr. P. D. STRAUSBAUGH of the Department of Botany, and to Dr. R. B. DUSTMAN of the Department of Agricultural Chemistry, in whose excellently equipped laboratory and with whose helpful advice the first extracts were made and tested. Thanks are due also to the administrative officers of West Virginia University for many courtesies and privileges extended to facilitate the work. Repetitions and extensions of the work were made in the laboratories at the University of Chicago during the years since 1928.

Materials and methods

Two carbohydrate-high and two protein-high seeds were chosen for the investigation. Japanese buckwheat and amber sorghum represented the first group; and mammoth clover and the Arlington cow pea represented the second group. An abundant supply of these seeds was furnished by the Department of Agronomy from their stock supplies. The seeds were first ground coarsely in a feed mill, but the Merker mill was relied upon for the final stages of grinding. It was found possible to reduce the material with this mill, even the tough hulls of buckwheat seeds, to 100-mesh size. As the mammoth clover seed was found to offer special difficulties because of its colloidal properties when ground to this degree of fineness, later work was done with it ground only to 60-mesh size. The freezing point depressions were measured by standard Beckmann apparatus, using either a salt-ice mixture or ether bath for freezing.

Extracts were made either by extracting the seed substances with cold water (32°–34° F.) or with hot alcohol. The earlier work was done with cold water; but it was found that with this method the reducing sugars in the extract were always larger in quantity than the non-reducing ones. As this result was not in accord with the results usually obtained when alcoholic extractions are made, the alcoholic extraction method was used as a check upon the water extraction method. It was felt that this would throw some light upon the amount of autolysis that can still occur at low temperatures (diastatic action, etc.). It will be evident later, when the data are presented, that the cold water extracts were made under conditions that seem to permit a degree of enzymic decomposition.

In making the cold water extracts, 100 gm. of the ground seed material, thoroughly mixed, was placed in a 1000 cc. Erlenmeyer flask with 500 cc. of distilled water. These flasks were kept in a cold storage room with temperature just above freezing. The mash was shaken up twice daily from May 8 to May 13. At the close of the extraction period the clear extract was decanted on to a filter (Whatman no. 2 paper with platinum filter cones). The extracting material, handled at all times in the cold room, was washed repeatedly with 25 cc. portions of water (all carefully drained, decanted, and filtered), until at the close of the washings there was exactly a half-liter of extract from 100 gm. of material. The only departure from this procedure was used with mammoth clover seed extracts. These refused to filter because of their great viscosity. It was necessary, therefore, to resort to centrifuging to remove the solids from suspension in this case. This method is not considered as efficient as filtration in the case of strongly viscous solutions. The centrifuging was done in portions at about 1800 r.p.m. The washing and centrifuging of the extract was continued until there was 1 liter of extract from the original 100 gm. of seed powder.

The extracts were then analyzed for three constituents:

1. Total dry matter and total ash from an aliquot of each extract were determined by gravimetric means. The ashing was done in an electric muffle furnace in broad platinum dishes.

2. From other aliquots of the extracts, cleared of colloids by neutral lead acetate and deleaded with potassium oxalate, determinations were made of reducing and non-reducing sugars. In some of the determinations, gravimetric methods (copper reduction) were used, taking every precaution possible to obviate the usual sources of error in gravimetric procedures. In other cases, the SHAFFER-HARTMAN iodometric method was used. In particular, the reducing power of the alcoholic extracts was determined by the micro SHAFFER-HARTMAN sugar method (STILES ET AL. **6**). Inversion of the non-reducing sugars was carried out with 1 per cent HCl at the temperature of the boiling water bath for 2 hours. The difference between the reducing power after inversion and that before inversion is considered a measure of the non-reducing sugars. Mammoth clover seed extracts produce a gelatinous precipitate at the moment of addition of

the alkaline constituent of Fehling's solution. As this interfered with gravimetric determinations of reducing power in these extracts, the iodometric method was used instead of gravimetric determinations in this case. Careful tests made with known quantities of sugars, used separately and with the extract, showed that the method was sufficiently accurate with reference to the sugar recovered.

3. Total nitrogen of the extracts was determined by drying an aliquot (duplicates) in a Kjeldahl flask on a water bath, after which the salicylic modification to preserve nitrate nitrogen was used, with Hg (5) as catalyst, and with cochineal indicator in the final titration. The results of many of the measurements made are recorded in the following section. It is not practicable to attempt to present more than a fraction of our data, which are limited to the needs of the discussion.

Results

Freezing point depressions

The freezing point depression obtained from freezing a mixture of seed substance in powdered form with water will depend upon the degree of dilution employed. In table I are shown some of the depressions observed with the four seeds used, and some of the variations which were noted when the amount of water was varied in the case of the Arlington cow pea meal, and with the viscous suspensions of mammoth clover seed powder.

In the case of the Arlington cow pea, the electrical conductivity of the various dilutions was measured. The results, expressed in reciprocal ohms (mhos), are given in table II.

The changes in osmotic concentration (freezing point depression) and conductivity by dilution of the seed substance with water are shown graphically in figure 1. As is to be expected, the conductivity and freezing point depressions decrease with dilution, but the relations are not linear. The more dilute solutions have relatively greater conductivity and greater depression of freezing points than the more concentrated ones. This is probably partly to be explained by a more complete ionization of the electrolytes in the dilute solutions, or to the greater degree of "activity" of the ions in the greater dilutions.

The data of table I confirm in a general way the findings of Bouyoucos and McCool, that ground seeds mixed with water produce solutions of concentration sufficient to cause marked depression of

TABLE I

FREEZING POINT DEPRESSIONS OBTAINED WITH MIXTURES OF SEED SUBSTANCE AND WATER

Variety		Weight of seed (gm.)	Water (cc.)	Thermometer zero (°)	Freezing point* (observed) (°)	Depression* (°C.)	Osmotic pressure (atm.)
Black amber sorghum	I...	10	40	5.130	5.011	0.119	1.435
	II..	10	40	5.130	5.015	0.115	1.387
Japanese buck wheat	I...	10	40	5.130	5.010	0.120	1.447
	II..	10	40	5.130	5.010	0.120	1.447
Arlington cow pea	I...	10	20	5.130	4.454	0.676	7.866
	II..	10	20	5.130	4.505	0.625	7.529
	I...	10	40	5.130	4.818	0.312	3.761
	II..	10	40	5.130	4.820	0.310	3.737
	I...	10	60	5.130	4.935	0.195	2.351
	II..	10	60	5.130	4.940	0.190	2.291
	I...	10	80	5.130	4.970	0.160	1.930
Mammoth clover	I...	10	20†	5.910	5.643	0.267	3.221
	II..	10	20†	5.910	5.644	0.266	3.209
	I...	10	80	5.130	4.954	0.176	2.134
	II..	10	80	5.130	4.956	0.174	2.098

* Figures in these columns are averages of three or four determinations differing usually not much more than 0.002° C.

† Mammoth clover seed 10:20 was so pasty that good stirring was not possible. The depression values are believed not to be very accurate at this concentration, possibly involving water only in the interface between the thermometer and the seed mass. At this concentration the depression should probably be much greater, as indicated by the cow pea data.

TABLE II

Seed substance (gm.)	Water (cc.)	Conductivity (mhos)
10....................	20	0.002974
10....................	40	0.001676
10....................	60	0.001174
10....................	80	0.000950

the freezing point. In none of our measurements, however, did we find as high depressions as were reported by these investigators in certain cases. It may be remarked that it is very easy to exaggerate

the depression of the freezing point by having the bath too much colder than the material being tested. The bath must be kept only slightly below the material in temperature, regardless of the greater time it takes for a measurement, or the heat of freezing will not register its full amount. We took extreme care in our work not to make the energy gradient too great. But differences in preparation, and

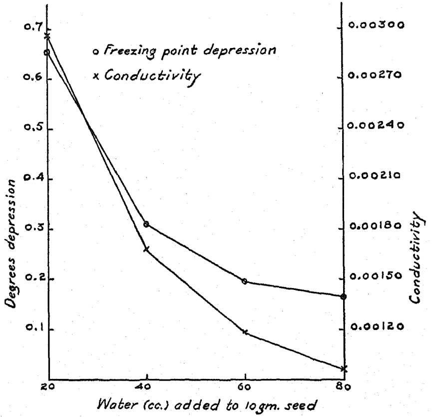


FIG. 1.—Changes in freezing point depression and conductivity with dilution

differences in material used, are probably more responsible for our somewhat lower values.

An effort was made to determine whether the time elapsing between the addition of the water to the seeds and the determination of the freezing point would make any notable changes in the values obtained. Two series of tests were made with the Arlington cow pea (10:40) which show that the soluble constituents go into solution rather rapidly, or at least become able to affect freezing point depressions, and that the concentration does not increase materially on standing, at least during the first few hours. On long standing, of

course, autolytic decompositions would make a difference. But whether a measurement is made after a few minutes or after a couple of hours makes only a rather small difference in the results.

The data for two of these samples are summarized briefly in table III. Sample I shows a slight tendency to change with time, but sample II remained practically constant for five hours. Slight inequalities in the samples chosen for the tests or imperfect mixing might rather easily produce results as irregular as they appear in

TABLE III

	Sample I		Sample II	
	Thermometer zero	Freezing point	Thermometer zero	Freezing point
Immediate	5.130	4.663	5.130	4.505
1 hour	5.130	4.460	5.130	4.505
3 hour	5.130	4.460	5.130	4.502
5 hour	5.130	4.430	5.130	4.505

sample I. We are inclined to think that there is prompt solution of such materials as are soluble, and that only after some hours of standing do the diastatic and other autolytic fermentations disturb the relations seriously.

Total solids

The analyses for total solids were run in duplicate on two different sets of samples, prepared at different times and places, and probably under unappreciated or unrecognized differences in handling. About the same technique was used in preparing the samples, except that the temperatures could not be made identical in the two cases, a very important variation, even if the differences were small. This could not be avoided. Another important difference was the mesh size of one of the samples. In the case of mammoth clover, the first extract was made from 100-mesh seed powder; the second was ground only to 60-mesh because the first one was too difficult to handle, too viscous, too hard to clear. While these differences in method make considerable difference in results, they cannot be said to vitiate the general conclusions which may be drawn from the investigation. It is

obvious from the large discrepancy in the mammoth clover data (table IV) that the 100-mesh material could not be cleared as completely by centrifuging as was the 60-mesh sample. That this is not the whole explanation, however, is evident from the difference between mammoth clover I_1 and I_2, table IV. Sample I_2 was taken from a solution which had passed through the Whatman no. 2 filter, while sample I_1 was pipetted from the perfectly clear limpid supernatant liquid which had settled completely, but had refused to filter. While sample I_1 might have contained, and probably did contain, some undissolved colloidal aggregates, sample I_2 could hardly have done so. Evidently the fineness of grinding here increased the solubility in sample I_2 about 6 per cent above samples III_1 and III_2, almost a 40 per cent increase in solubility.

In the case of the other seeds, the duplicate determinations from individual runs check closely enough, but the different runs differ from unknown causes. It seems best to record the data and average the values of the determinations (table IV). The figures indicate at least the general order of solubility, and the data throw a certain amount of light on the problems of solubility of seed substance when in a fine state of subdivision.

The data in table IV indicate that a very considerable portion of the substance of a seed may go into solution when it is extracted for a period of five or six days with water. It should be stated, also, that there were never the slightest indications of bacterial or mold growth in any of the flasks during preparation of the extracts. A check on the stability of carbohydrates away from their hydrolytic and respiratory enzymes was obtained from unsterilized standard sugar solutions that were placed in the cold chambers in February and kept until June. Polarized at the start of the tests, they were polarized again in June, and showed no measurable change in sugar concentration during four months of storage at 32°–34° F. There is in our extracts strong evidence of the occurrence of autolytic changes with time. No doubt this should have been expected, since invertase activity is not prevented at zero, and many of the enzymes may act slowly at this temperature. The substances that cause freezing point depression go into solution quickly, and give as large depressions in a few minutes as they do in a few hours; but on long standing, autol-

ysis would certainly cause an increasing amount of carbohydrates to appear in the extracts. Proteins may also be affected, but the change would be in a direction to favor the BOUYOUCOS-McCOOL suggestion rather than to discredit it.

TABLE IV

TOTAL SOLIDS OF SEED EXTRACTS IN PERCENTAGE OF ORIGINAL DRY WEIGHT

Seed extracted		Original dry weight (%)	
Black amber sorghum	I_1	4.66	
	I_2	4.64	
	II_1	8.08	
	II_2	8.02	
Average		6.36	
Japanese buckwheat	I_1	9.86	
	I_2	9.94	
	II_1	11.74	
	II_2	11.53	
Average		10.77	
Arlington cow pea	I_1	16.79	
	I_2	16.93	
	II_1	18.98	
	II_2	19.01	
Average		17.93	
Mammoth clover	I_1	24.50	100-mesh
	I_2	21.75	100-mesh
	II_1	24.49	100-mesh
	II_2	24.48	100-mesh
	III_1	15.91	60-mesh
	III_2	15.33	60-mesh
Average (all)		21.08	

TOTAL SOLUBLE CARBOHYDRATES

Although analyses of seeds usually show the presence of some carbohydrates in soluble form, it is usual to find the non-reducing sugars more abundant than the reducing sugars.

Several years ago MACGILLIVRAY (4) reported analyses of tomato seeds from fresh tomato pulp. He found from 3.23 to 3.70 per cent of free reducing sugars (dry weight basis), calculating them as glucose. These seeds had not been allowed to dry after removing them from the pulp, nor were they washed free of sugar before preserving in alcohol, a circumstance that might favor the presence of a larger amount of free reducing sugar than is usual in seeds when thoroughly washed and dried. KRAYBILL (2) reports that sucrose occurs in wheat,

varying in quantity in the different varieties, the percentages being as follows: Shepherd 1.61, Gladden 1.53, Fulhard 1.52, Nittany 1.65, Purkhoff 1.55, Kawvale 1.53, and Fultz 1.25 per cent. He also states that almonds, castor beans, and cucumber seeds contain from 1 to 2 per cent sucrose; and that he has isolated sucrose pure from soy beans, which contain a considerable amount of this sugar. It is obvious that one cannot assume the absence of sugars in dry seeds.

Our extracts, which were made with prolonged contact with cold water, indicated, as has been stated, that the reducing sugars were more abundant than the non-reducing types. This is frankly accepted as evidence that, in spite of the near zero temperatures, some autolytic changes occurred that increased the quantities of sugar determined. That we might not overestimate the rôle of soluble carbohydrates in the depression of freezing points of seed extracts, some hot alcoholic extracts were made so that comparisons might be made with the water extracts. The results are presented in tables V and VI. Table V gives the data for the cold water extracts.

TABLE V

TOTAL, REDUCING, AND NON-REDUCING SUGARS IN COLD WATER SEED EXTRACTS (DRY WEIGHT BASIS)

Seed extracted		Reducing sugar (%)	Non-reducing sugar (%)	Total soluble sugar (%)
Black amber sorghum	I	1.58	1.31	2.89
	II	1.57	1.36	2.93
Japanese buckwheat	I	2.18	0.12	2.30
	II	2.08	0.16	2.24
Arlington cow pea	I	5.32	0.75	6.07
	II	5.29	0.84	6.13
Mammoth clover	I	1.91	1.48	3.39
	II	1.84	1.49	3.33

The smaller total quantities of sugars were found in the carbohydrate-high seeds, such as sorghum and buckwheat; and somewhat larger amounts were found in the protein-high seeds, mammoth clover, and particularly in the Arlington cow pea. It was the unusual amount of reducing sugars in the cow pea, and the uniform predominance of the reducing sugars over the non-reducing, that led to the knowledge that autolysis was an important factor when seeds are

extracted for long periods in the cold. The hot alcoholic extractions yielded very low results for both reducing and non-reducing sugars, certainly not so high as many other seed analyses would lead one to expect. The results are given in table VI. These determinations were made by the iodometric method (micro SHAFFER-HARTMAN), with 1.5 gm. samples (100-mesh or 60-mesh) which were extracted with 80 per cent alcohol for three hours. The figures are in each case the average of four determinations. From these data it seems that sugars are either much less abundant in seed extracts than the data from cold water extractions would lead one to believe, or that they

TABLE VI

TOTAL, REDUCING, AND NON-REDUCING SUGARS IN HOT ALCOHOLIC SEED EXTRACTS (DRY WEIGHT BASIS)

SEED EXTRACTED	REDUCING SUGAR (%)	NON-REDUCING SUGAR (%)	TOTAL SOLUBLE SUGAR (%)
Black amber sorghum	0.08	0.17	0.25
Japanese buckwheat	0.07	0.07	0.14
Arlington cow pea	0.18	0.19	0.37
Mammoth clover	0.33	0.11	0.44

do not extract readily with hot alcohol. Probably autolysis has multiplied the sugars in solution in the case of the data in the last column of table V.

Nevertheless, from all of the facts presented it appears that there are sugars in dry seeds; and that while the quantities may be small, rarely exceeding 2 per cent, and frequently falling below that amount, yet they are not negligible in the problem of freezing point depressions of seed extracts. Especially are they not negligible when the freezing point depressions are small.

TOTAL ASH

The dried aliquots used in obtaining total solids in solution were carefully ashed at low red heat to avoid serious volatilization of bases. The different extracts again showed some variations. Duplicates from a single extract checked closely; but evidently the details of preparation would have to be very closely standardized to secure

concordant results from different extracts. The data on total ash found in the various extracts are presented in table VII. They give a fair measure of the total ash elements which occur in such seed extracts.

These results (table VII) show the presence of from approximately 1 per cent to nearly 3 per cent of ash in the form of oxides of the

TABLE VII

TOTAL ASH DETERMINATIONS ON SEED EXTRACTS (DRY WEIGHT BASIS)

Variety of seed		Original sample (%)
Black amber sorghum	I_1	0.956
	I_2	0.952
	II_1	1.34
	II_2	1.35
Average		1.15
Japanese buckwheat	I_1	1.286
	I_2	1.282
	II_1	1.280
	II_2	1.270
Average		1.28
Arlington cow pea	I_1	2.82
	I_2	2.91
	II_1	2.58
	II_2	2.55
Average		2.75
Mammoth clover	I_1	2.686
	I_2	2.684
	II_1	1.58
	II_2	1.55
Average		2.12

basic elements. While the results are somewhat variable, they establish a sound basis on which to estimate the ash influence in the freezing point behavior of finely ground seeds.

Total nitrogen of extracts

Total nitrogen was determined by methods already stated, and the results were used in calculating the amount of protein in the extracts. No attempts were made to fraction the nitrogen, and total $N \times 6.25$ is accepted as a conventional approximation of the quantity of protein dissolved in the extracts. In table VIII are shown the total nitrogen determinations, and the corresponding calculated percentages of protein present.

It is seen by examination of the data that the results with the protein-high seeds are not at all comparable in the two sets of extracts. In one instance the extract of mammoth clover seed contains 50 per cent more protein in solution than the other extract. Here again it is the extract from 100-mesh material that runs higher than the determinations from 60-mesh powder. The differences in the cow pea

TABLE VIII

TOTAL NITROGEN DETERMINATIONS ON SEED EXTRACTS (DRY WEIGHT BASIS)

Variety of seed		Soluble nitrogen in extracts from original sample (%)	Soluble protein in original sample (%)
Black amber sorghum	I_1	0.032	0.20
	I_2	0.037	0.231
	II_1	0.0256	0.160
	II_2	0.0248	0.155
Average		0.299	0.187
Japanese buckwheat	I_1	0.258	1.613
	I_2	0.262	1.638
	II_1	0.267	1.669
	II_2	0.266	1.663
Average		0.263	1.645
Arlington cow pea	I_1	0.96	6.00
	I_2	0.96	6.00
	II_1	0.329	2.056
	II_2	0.329	2.056
Average		0.645	4.028
Mammoth clover	I_1	0.65	4.063
	I_2	0.67	4.188
	II_1	1.0184	6.365
	II_2	1.0074	6.296
Average		0.836	5.228

data are not easily explained. The extracts were made in the same manner, and so far as known, all of the procedures were essentially the same; but one set runs nearly three times as much soluble protein as the other. There is fair agreement in the case of Japanese buckwheat, and both sets of analyses agree in the small amount of proteins occurring in the extracts of black amber sorghum. It is not so much absolute quantities and close agreements that are needed for this problem, however, as general notions of the amount of proteins that are soluble in such seed powders. The data give such approximations, regardless of the irregularities which they show.

We have now given such approximations for carbohydrates, ash elements, and proteins. When the totals of these are compared with the total solids of the extracts, they by no means account for all of the soluble materials. For example, in the extract of the Arlington cow pea, one of these analyses shows 18.98 per cent total solids; of this total, 16.40 per cent is organic and 2.58 per cent inorganic. The total sugars account for 6.07 per cent of the organic material, and the dissolved proteins account for 6.0 per cent. The remainder is not determined. We are not trying to make a complete determination of all of the soluble material, but merely to establish the general situation as to the main soluble constituents, salts, sugars, and proteins. With the facts at hand it is possible to consider intelligently the problems of freezing point depressions as observed in such seed powders when frozen with definite quantities of water.

Discussion

The data presented in the preceding section of this paper we believe indicate clearly that there are appreciable amounts of both ash elements and carbohydrates in the substance of seeds, and that these substances are capable of going into solution quickly when the finely divided substance is immersed in water. The matter that interests us mainly, however, is whether there is a large enough quantity of these materials present to account for the depression of freezing points that are observed in such extracts or mixtures of seed powder and water when frozen, and to produce the osmotic concentrations which these depressions indicate (1.5–8.0 atm.), as shown in table I.

It is not necessary to enter upon any lengthy discussion of this subject; an example will serve to make clear the significance of our results. Take, for example, the case of the Arlington cow pea, in which case a 10:40 mixture of seed substance and water gave an observed depression of freezing point of 0.312° C., corresponding to an osmotic concentration of 3.761 atm. (table I). The data of table VII show that the average amount of ash for four samples of this seed was 2.75 per cent; and from two analyses in table VIII, the sugars were found to be 6 per cent. As has already been suggested, these sugar figures may be too high; but how much too high must be left to others to determine. The evidence from hot alcoholic extractions

can hardly be applied, since all freezing point depressions are measured in cold water mixtures with ample time for solution. At any rate, the treatment of this example illustrates the reasoning to be applied to any such case, whether the sugar data in this particular instance are reliable or not.

What do these figures of ash and sugar concentration mean in terms of freezing point depression? In the first place, if there are 2.75 per cent of ash elements present, the 10 gm. sample of powder will contain 0.275 gm. of ash. Furthermore, since the mixture was a 10:40 one, there is but one twenty-fifth of a liter of water present. The ash is present, then, at the rate of 25×0.275, or 6.875 gm. per liter. If one were certain of the average weight of the ions present, it would not be difficult to determine about how much depression of freezing point should result from the action of the dissolved salts. We do not know the average weight of the ions, nor do we know the extent to which they are ionized in the seed substance when immersed. If the ash ions weighed on the average about as much as Ca, K, Mg, etc., it would require only 35 or 40 gram-ions per liter to account for a depression of $-1.86°$ C., and a corresponding osmotic concentration of 22.4 atm., supposing complete ionization. Under such circumstances, 6.875 gram-ions per liter would be equivalent to about one-sixth molar solution, and should cause a freezing point depression corresponding to an osmotic concentration of about 3.7 atm. This is as much as the entire depression observed in the case of the Arlington cow pea. But, it may be argued that these basic ions are not paired with simple inorganic anions, but accompany complex organic anions of protoplasm and other structural ingredients which are not osmotically active. In that case the weight of ash is greater than the actual weight of inorganic ions in the tissues; for in the ash they are all oxidized, and we weigh the added oxygen as a part of the ash. Of course there are some simple inorganic anions in the tissues, chloride, sulphate, phosphate, nitrate, etc., but still it is probable that ash is considerably heavier than the real weight of inorganic ions in the tissue. It would be more than generous, however, to allow as much as 50 per cent of the weight of ash for the increase in oxygen. If we estimate that the actual weight of the ions is only half of the observed weight, then we would have to halve the

effects of the salts, and instead of crediting them with the entire osmotic action (in table I there is only 3.761 atm. for which to account), we would have to attribute possibly 1.85 to 2 atm. to the salts alone. This is discounting them very liberally.

The carbohydrate situation is equally interesting. In table III it is shown that there is 5.3 per cent of reducing sugar and 0.8 per cent of non-reducing sugar in the cow pea extracts, or about 6.0 per cent total sugar. This means that a 10-gm. sample would contain 0.6 gm. of sugar, or at the rate of 15 gm. per liter in a 10:40 mixture. Such a solution, if composed of hexose reducing sugars only, would possess the osmotic concentration of a twelfth-molar solution, equivalent to 1.8 atmospheres. It is of course actually less, since some of the sugar is of non-reducing type, with heavier molecules. Moreover, it is entirely possible, or even probable, that these figures are too high. But even a 2 per cent soluble sugar content would bring about a depression of freezing point equivalent to a concentration of from 0.3 to 0.6 atm., depending on whether it were sucrose or dextrose.

The figures obtained from hot alcoholic extracts would give almost no influence to sugars. But since our freezing point tests were all made in aqueous mixtures, we are using the figures obtained from aqueous extracts for our arguments.

It must be obvious from the data that almost the entire freezing point depression caused by the Arlington cow pea meal when frozen in water is accounted for by our data on salts and carbohydrates. It would seem fair to make the claim that in a general way these two factors are most largely responsible for the freezing point depression. There are, of course, other soluble substances present which have not been determined, and which must play their part.

Finally, the proteins should be mentioned briefly. The data in table VIII indicate roughly the presence of from 0.15 to 6.3 per cent of soluble protein in the various extracts as made. If the nitrogen really represents proteins, and not amides and amino acids, their effects on freezing points would have to be utterly negligible. Even with 6.3 per cent protein, a 10 gm. sample would contain but 0.63 gm., or at the rate of 15.75 gm. per liter of water in a 10:40 mixture. This amount would represent about one two-thousandth of the

gram-molecular weight of a light weight protein; and this concentration should theoretically produce a freezing point depression corresponding to an osmotic concentration of 0.011 atm. (about 0.001° C. of depression).

It is concluded, therefore, that the depression of freezing points, and the osmotic concentrations shown by finely powdered seed substance when frozen with water, are caused by the salts and sugars, which are present in sufficient quantity to account satisfactorily for the observed phenomena. There seems to be no reason for ascribing to proteins any special rôle in connection with the osmotic action of seed substances. They are undoubtedly as important in connection with imbibition phenomena as any other colloidal constituents of the seeds.

Summary

1. Freezing point depression determinations were made on mixtures of finely powdered seeds and water. The four varieties of seeds used were black amber sorghum, Japanese buckwheat, Arlington cow pea, and mammoth clover. These determinations, repeated many times, confirm in a general way the measurements of Bouyoucos and McCool, but with lower values.

2. Some conductivity measurements were made to throw light upon the effects of dilution on the electrolytic constituents of the seed extracts.

3. Analyses of cold water extracts of the seed powders are presented, covering total solids, total soluble carbohydrates (reducing and non-reducing sugars), total ash, and total nitrogen (proteins).

4. These analyses, coupled with analyses of alcoholic extracts, indicate that autolysis occurs even at temperatures but slightly above freezing.

5. Rough calculations have been made, using the values obtained in the analyses for ash, sugars, and proteins, to determine the approximate influence of each of these substances in producing freezing point depressions and osmotic action.

6. From these calculations it is concluded that almost all of the freezing point depression can be accounted for by the quantities of salts and sugars present.

7. There are other unknown and undetermined soluble constituents present in the extracts, whose action must also play a part in freezing point depression and osmotic action.

8. The proteins are practically negligible as a factor in the freezing point behavior of seed extracts.

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DEVELOPMENTAL MORPHOLOGY OF ALLIUM CEPA

CONTRIBUTIONS FROM THE HULL BOTANICAL LABORATORY 448

CHARLES ANDREW HOFFMAN

(WITH TWENTY-NINE FIGURES)

Introduction

Of all the Liliaceae, the onion, *Allium cepa* L., is of greatest economic importance. Its distribution is world-wide and its popularity as an article of food is almost as extensive. SACHS (10), in a well illustrated article, discusses the microchemistry of the seed and seedling, together with the anatomy and germination of the seedling. SIDERIS (11) notes that onion roots emerge in two sets, one group for the early life of the seedling and another for bulb formation. The first roots die as the second group becomes functional. JONES and BOSWELL (5) found that the primordia of the flower axis in bulbs which were formed the previous season are differentiated during March. POESE (8) notes that the vascular bundles in all the Liliaceae he investigated fluctuate between a collateral and a concentric type, the latter appearing most commonly in the rhizome and at the nodes. MANGIN (6) states that the meristem giving rise to adventitious roots is absent in all monocotyledons without these roots.

MATERIAL AND METHODS.—In the fall of 1930 and in the following summer, material was grown from pedigreed seed of the Yellow Globe Danvers onion. From this material the various stages to be studied were selected. All the material, except the primary roots which were killed in Flemming's medium fluid, was killed in a mixture of four parts absolute alcohol and one part glacial acetic acid, passed through five mixtures of ethyl and butyl alcohol, and finally imbedded in paraffin as described by ZIRKLE (13). The primary roots were cut 6μ thick and the other material from 8 to 15μ thick.

Embryo

The mature embryo is a curved cylinder about 0.4 mm. in diameter and 6 mm. long, of almost uniform width, somewhat pointed

at the hypocotyledonary end and rounded at the opposite or haustorial end. It is almost completely surrounded by an endosperm whose cells contain fat (10), and whose walls are much thickened with a hemicellulose which on hydrolysis yields mannose (2). The haustorial end may be merely curved at right angles to the remainder of the embryo or may form more than a complete circle. A 270° arc is the average. About one-tenth of the length of the embryo is hypocotyl; the rest is cotyledon. Proximal to the tip of the hypocotyl, a distance twice the diameter of the embryo, is a slit in the cotyledon or first leaf through which the second leaf grows. This slit and the cavity inside it are usually filled with a waxy or gummy substance. The cavity thus formed by the hollow base of the cotyledon surrounds the primordium of the second leaf, which usually is tongue-shaped, with a length about twice its thickness. The slit, according to SACHS (10), occurs with equal frequency on the concave and on the convex sides of the embryo.

All of the cells of the embryo are very thin walled. The five regions of the embryo as seen in longisection and described by SACHS are clearly distinguishable. They are: (1) the peripheral layer, which a week after planting has formed the epidermis of the cotyledon together with its stomata and guard cells; (2) the parenchyma of hypocotyl and of cotyledon which, even in the embryo, has many intercellular spaces; (3) the single procambial strand running from the promeristem of the root tip to the stem region and up through the cotyledon to the peripheral layer of the haustorial portion. The cells of this procambial strand and of the parenchyma are in long rows. In some embryos the procambial strand contains spiral elements which are first differentiated above the region of the stem, a distance two or three times the diameter of the embryo; (4) the promeristem of the root and of the stem tip; (5) the root cap.

Germination

SACHS' account of the changes which occur in the form of the embryo and in the content of the cells during germination was confirmed. Development of the embryo begins with elongation of the lower and middle regions of the cotyledon. Within 24 hours the hypocotyl is pushed through the seed coat and soon thereafter most

of the cotyledon also is pushed out. The tip of the cotyledon, however, remains imbedded in the endosperm of the seed from which it absorbs food. When 4–6 mm. of the embryo is outside the seed coat, a bending in the cotyledon occurs above the slit so that the hypocotyl is directed downward. By the end of a week the root has grown downward 4–6 cm., while the short stem region has remained at about the same level as that occupied by the seed at the time of planting, and the cotyledon has attained a length of 8–12 mm. In the cotyledon, midway between the slit and the attached seed, a region has become sharply bent double so as to present its convex side to the soil surface above it. This is the soil-penetrating organ or "knee," which is carried upward by elongation of the part of the cotyledon on either side of it.

Apparently there is no localized meristematic region in any part of the cotyledon, cell divisions occurring throughout its entire length, although the cells at each end reach their full size later than the others. After becoming vacuolate and six to eight times their embryonic length, the cells of the cotyledon divide not more than two or three times and elongate slightly. During the first five days no mitotic figures are found in the cotyledon, the growth being due to cell enlargement alone. A few days later figures become abundant but two weeks later are absent. Mitoses occur in all tissues but last to begin is the region near the seed. After eight or ten days, elongation of the seed limb of the cotyledon ceases, while the stem limb, especially near the stem region, continues to elongate slightly. This causes the stem limb to be curved and the seed limb to be stretched across the curve like a bowstring. By this time the haustorium has absorbed so much of the endosperm that it lies loosely in it, and, under the growth tension of the cotyledon, the haustorium is pulled out of the seed and germination is completed. At this time the cells in the concave side of the knee grow until finally the seed limb of the cotyledon also points upward, although a bend always marks the location of the knee. The haustorium, once out of the seed, immediately withers but the rest of the cotyledon remains photosynthetic.

By the time germination is completed the primary root has attained almost its full length of 10–12 cm. It does not branch, and all subsequent roots originate adventitiously from the stem. Just

above the region of root hairs at the top of the hypocotyl the first two or three adventitious roots push out through the outer cortex of the lower stem. This action seems to be both lysigenous and schizogenous. These roots originate from the parenchyma of the inner cortex, which becomes meristematic (fig. 11). The first of these roots arises from the cortical sector below the slit. The next appears usually more than 90° to the right or left of the first, and if there be a third, it is developed in the region diametrically opposite the second. Subsequent adventitious roots originate as does the first. There is no regularity in the order or place of their appearance. Each successive root is usually less than half its own diameter above the previous one. A plant five months old may have had well over 100 root primordia and roots.

Primary root

The primary root is an exarch radial protostele, usually diarch but sometimes triarch. When both xylem and phloem are mature there is no parenchyma in the stele except the uniseriate pericycle. The central metaxylem consists of one to five, usually two, larger elements which mature into scalariform vessels (fig. 9). Casparian strips are apparent in a seedling six days old, but never become very thick. Cortex and epidermis are parenchymatous.

Development of the root axis is like the "type 2" of JANCZEWSKI (4) as modified by TREUB (12). The meristem consists of two regions, the one giving rise to the plerome and the other to the periblem, epidermis, and root cap (fig. 15).

As seen in longisection the stele appears to arise from two, three, or four cells at its distal end. From the derivatives of these a pericycle is first differentiated, and then one of the centrally located cells enlarges and thereafter does not divide so often as those surrounding it but grows in all dimensions, especially longitudinally. From a row of such cells the large central vessel is formed. One or two periclinal divisions of the surrounding cells bring the stele to its diameter of five to ten, usually seven, cells. Subsequent cell divisions take place transversely and thus longitudinal growth continues. The cells of the pericycle are slightly shorter than those of the endodermis and neighboring cortical cells, but are more easily distin-

guished because they are immediately outside the very slender procambial cells.

Distal to the stelar histogen is a meristematic region three to six cells in diameter and two to four cells deep. From the derivatives of the peripheral cells of the upper layer or layers of this region the cortical cells are differentiated. From the derivatives of a lower layer of the same region is differentiated the single layer of epidermal cells which undergo no further periclinal divisions but divide only transversely and anticlinally. From the lowest layers of this meristematic region the root cap is differentiated. The cortex, epidermis, and root cap are first distinguishable as distinct regions at about equal distances from the center of the common histogen from which they are derived. One or two periclinal divisions of the cells of the region between the epidermis and stele give the cortex its mature width of from five to eight, usually six cells. The cortical cells have divided transversely more frequently than those of the stele and less frequently than those of the epidermis, and thus are intermediate in length between the two.

The root cap consists of several conical layers of cells of which the outer is oldest and largest. Near the apex of each cone the cells are larger, slightly more numerous, and elongated longitudinally, resulting in the wedge-shaped outline of the cap. At the center of the root cap, where there has been least distortion by growth, a longisection shows two to four somewhat irregular vertical rows of cells, each row still clearly traceable to a single cell of the histogen.

Vascular anatomy of cotyledon

Differentiation and maturation of xylem first begin above the slit and from this point proceed upward and downward at about equal rates. The protoxylem in the cotyledon, and other leaves as well, consists usually of spiral elements, although some annular thickenings may be present, especially in the tapering ends of the tracheids. In the cotyledon as seen in transection there is developed one protoxylem area and a band of metaxylem on each side of the younger part of this protoxylem. Eventually there is formed a stocky Y-shaped area of xylem with an accompanying area of phloem at the end of each upper arm of the Y (figs. 3–6). Sometimes

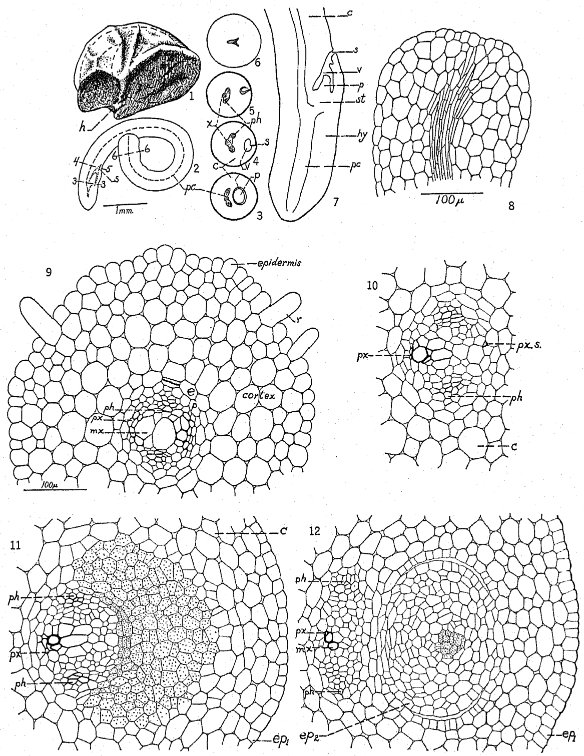
the xylem is separated into three or four groups by parenchyma, or the Y may be flattened like a short-stemmed T. Phloem matures first at the points farthest removed from the protoxylem.

In two seedlings, each with two growing points at the tip of the stem, there was only one cotyledon but two cavities, one for each growing point, and one vascular bundle in the cotyledon between the two cavities. The xylem of this bundle appeared in transection as an oblong band flanked on either side by a cavity with its growing point. The protoxylem was at the middle of one of the longer sides of the xylem area and a phloem group was at each of its ends. Thus in this case as well as normally development of the xylem was exarch. In one of the seedlings the primary root had four phloem groups and was probably tetrarch. The root of the other was not seen.

CHAUVEAUD (1) reports resorption of the protoxylem and its replacement *in situ* by metaxylem in the cotyledon of seedlings eight to ten days old. He figures ten transections but no longisections to show this. Actually no resorption occurs, but the protoxylem vessels become so stretched by the growth of the surrounding cells that their annular thickenings are so widely separated or their spiral elements so much stretched that the walls between thickenings collapse, and in transection the vessels are seen as only a very small patch of cellulose easily overlooked. This protoxylem becomes more difficult to find as the cotyledon becomes older. EAMES and MACDANIELS (3) report a similar situation in *Lobelia*. In longisection protoxylem is easily seen in all material less than four weeks old, at which age the second and third leaves have appeared and the cotyledon begins to wither from the tip downward toward its base. Development of the cotyledonary xylem is exarch throughout.

Anatomy of stem

In the germinating seedling all of the hypocotyl is rootlike in structure. Near the top of it the two protoxylem areas of the stele of the root appear rather abruptly, much separated from each other. The one extends into the cotyledon while the other ends at a distance equal to from one-third to one-half the diameter of the cotyledon beneath the primordium of the second leaf (fig. 7). The protoxylem of this latter strand is never extended up farther but ends blindly (fig. 10). Immediately above the level at which this protoxylem



FIGS. 1–12.—Fig. 1, seed showing position of embryo (drying of endosperm causes irregular wrinkling). Fig. 2, embryo dissected out of seed. Fig. 3, diagram of transection through embryo at level 3–3 of fig. 2. Figs. 4, 5, same, but at level 4–5, showing variation in structure at slit (*s*) and in outline of procambial strand (*pc*). Fig. 6, same, but at level 6–6. Fig. 7, diagram of longisection through portion of embryo. Fig. 8, longisection through tip of haustorium showing procambial cells extending to epidermis which is without a cuticle (*c*, cotyledon; *h*, hilum; *hy*, hypocotyl; *p*, leaf 2 primordium; *pc*, procambial strand; *ph*, position of future phloem; *s*, slit; *st*, stem; *v*, cavity in hollow base of cotyledon; *x*, position of future xylem). Fig. 9, transection through upper part of primary root of 6-day seedling. Fig. 10, transection 390μ above fig. 9, showing protoxylem under stem (*pxs*) consisting of single cell only. Fig. 11, transection 90μ above fig. 10, showing actively dividing cells of cortex (stippled) beginning formation of first adventitious roots. Fig. 12, transection 190μ above fig. 11, showing leaf 2 with first procambial strand (stippled) inside cotyledon (*c*, cortex; *e*, endodermis ep_1, epidermis of cotyledon; ep_2, same of second leaf; *mx*, metaxylem; *p*, pericycle; *ph*, phloem; *px*,

area ends, all of the xylem of the cotyledonary bundle and of the stem develops in one direction, away from the protoxylem of this cotyledonary bundle toward the center of the stem and even beyond it. At this level the four or five elements of the cotyledonary protoxylem are arranged side by side in a line which would pass perpendicularly to a radius of the stem extended through them. The metaxylem between this line of protoxylem and the point above the other protoxylem area appears in transection as a wedge-shaped area with two or three isolated metaxylem vessels at its point, but separated from the remainder of the metaxylem by parenchyma. Excluding the cotyledonary bundle, these isolated vessels are the first of the stem xylem to mature. Later at this level metaxylem matures on all sides of these isolated vessels, but at a slightly higher level, only on the side farthest from the cotyledonary protoxylem, that is, centrifugally. At the same time xylem matures also on each side of the metaxylem wedge as well as around its tip, but is separated from the wedge by a layer of parenchyma. Thus in transection there appears a thick U of xylem surrounding the wedge, except at its widest portion which is cotyledonary xylem. The base of this U is split off higher up to form the largest and central bundle of the second leaf. Part of the remaining arms form a portion of the two bundles of the second leaf, lateral to its largest bundle, and part continues upward to form the outer rather definite vascular layer of the stem (fig. 27). This outer layer, however, is formed largely from the steles of the adventitious roots.

The point at which parenchyma is first apparent in the upper hypocotyledonary xylem is arbitrarily called the bottom of the stem. In a seedling ten days old the stem is less than one-half as long as the diameter of the seedling and contains no matured xylem except the cotyledonary bundle. About ten more days are required for the first xylem to mature through this short length of stem up as far as the base of the second leaf. The lower part of this xylem consists of reticulate cells not more than three times as long as wide. The xylem slightly farther up is scalariform, while in the leaf the protoxylem is again of long spiral vessels which mature centrifugally.

In the center of the stem the vascular arrangement is determined largely by the manner in which the leaves develop. Each successive

leaf matures as an upright hollow cylinder, one side of which grows much more rapidly than the other, to form the blade, while the cylinder itself is the sheath surrounding the younger leaves and the apical meristem (figs. 24, 25). Because of the cylindrical sheath, therefore, the vertical vascular bundles of the leaf are arranged in a circle at its base. The largest bundle is near the center of the side forming the blade. The largest bundle of leaf 2 is farthest from the cotyledonary bundle and that of leaf 3 is near the cotyledonary bundle but separated from it by the sheath of leaf 2. In the stem the bases of the larger bundles of each leaf are connected with a horizontal U- or C-shaped ring of vascular tissue. Successively higher rings have vertical connections with each other, and thus a central parenchymatous area, the pith, is surrounded by a cylindrical network of vascular bundles. Lateral branchings extend from this central vascular cylinder toward the periphery of the stem, but are branched extensively both horizontally and vertically before they are anastomosed with the vascular layer limiting the stele. Over half of the stele is parenchymatous.

Leaf traces are never connected directly with the vascular tissue of the adventitious roots. The traces of larger bundles of the leaf are connected directly with the perimedullary vascular cylinder, while those of the smaller bundles are connected with the vascular tissue of the stem nearer its periphery. At the periphery of the stem the vascular tissue of the roots, with that of the outer stem, is spread out to form an almost continuous layer of intimately connected vascular bands outlining the stele of the stem. Leaf traces, as they pass through this layer of vascular tissue, are separated from it by parenchyma. The vascular connections of this peripheral layer with the perimedullary cylinder are never direct but irregular, and of such a nature that both soil minerals and organic foods may pass through a number of different routes to their destination. The bundles of the stem are for the most part amphivasal, with a layer or two of parenchyma separating the xylem and the phloem (fig. 27*B*). The amount of xylem on the side of the leaf trace farthest from the pith diminishes as the trace extends upward and outward, so that in the leaf the bundle is collateral.

A rather thick parenchymatous cortex penetrated by numerous

roots surrounds the stele. Since successive leaves touch each other, the stem is without an epidermis of its own. In longisection the stem appears somewhat heart-shaped, because successively formed parts have a greater diameter and because the apical meristem is increasing in size less rapidly than the surrounding area (fig. 27).

Adventitious roots

Adventitious roots originate very near the apical meristem of the stem and, because it is slightly sunken, at about the same level as this meristem (fig. 29). Their steles are continuous with that of the stem (fig. 28). Although the pericycle is not distinguishable as a single layer, it is in this region of the stem that the stele of the adventitious root originates. The cortex of the root appears continuous with the inner cortex of the stem. As the root elongates it first pushes through the outer cortical cells, which elongate somewhat during the process, and then through the bases of two or three fleshy sheaths. In so doing occasionally a root may penetrate the inner epidermis of a sheath but fail to pierce the outer. As a result the root usually grows upward for some distance through the mesophyll of the sheath, and finally dies when this becomes desiccated.

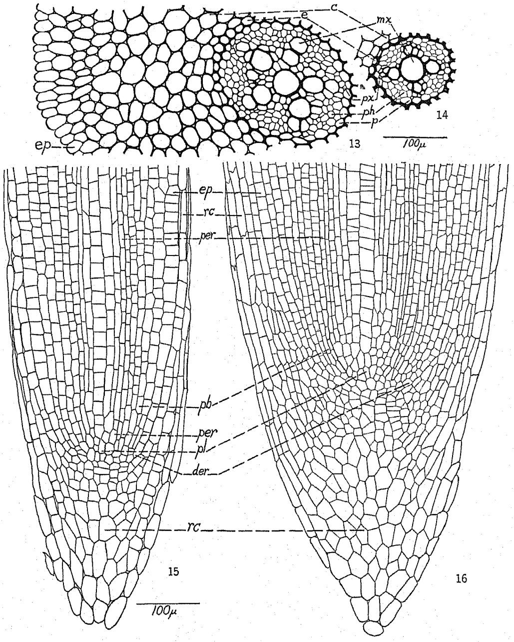
Development of the axis of the adventitious root proceeds exactly as in the primary root, except that in the former each region is larger because of a correspondingly greater number of cells (fig. 16). Tetrarch, pentarch, and hexarch steles occur on the same plant although the pentarch is most common (fig. 13).

In a mature root there is no space between the epidermis of its proximal part and the cortex of the stem through which it has broken (fig. 27). Adventitious roots may branch after they have attained a length of 10–15 cm. These branch roots break through the cortex and epidermis and usually occur on the convex side of the root. Older roots and leaves and older parts of the stem die and shrivel or disintegrate during the first year of a plant's development, thus making the bottom of the stem somewhat flat.

Development of leaf

The phyllotaxy of the onion is 1/2. The apical meristem of the stem from which leaves are formed, and which eventually grows up

into the flower scape, is a low, circular, dome-shaped mass of cells (fig. 18). One side of this region (*c*, figs. 17, 18) grows up more rapidly than the center or axis (*e*) or than the opposite side (*b*), and owing to



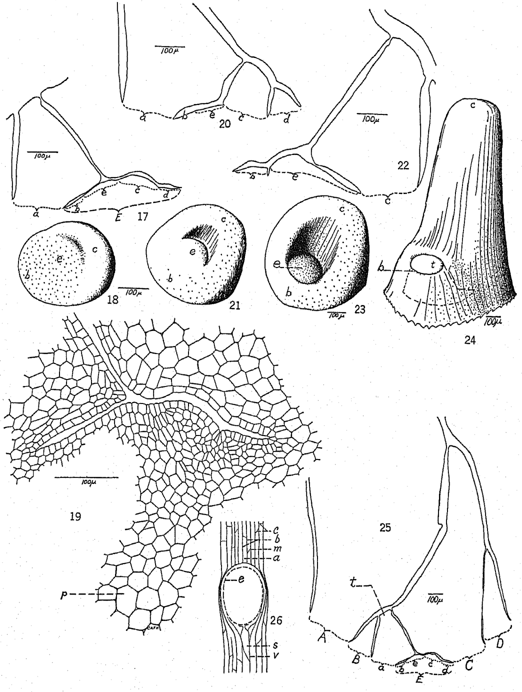
FIGS. 13–16.—Figs. 13, 14, transections of mature portions of adventitious roots: pentarch section (fig. 13) from portion of root near cortical region of stem; tetrarch (fig. 14) from portion 9 mm. distant from stem (*c*, cortical parenchyma; *p*, pericycle; *ph*, phloem; *px*, protoxylem). Fig. 15, median longisection of primary root grown in soil. Fig. 16, same of adventitious root grown in water (*der*, dermatogen; *ep*, epidermis; *pb*, periblem; *per*, pericycle; *pl*, plerome; *rc*, root cap).

centripetal growth as well, soon overgrows the axis (figs. 20–23). As the one side continues to grow higher, the entire periphery of the growing region begins to extend upward around the axis, from which there has now been differentiated the blade of the next leaf, which at this time is also growing at the same rate as the more slowly rising part of the periphery (figs. 24, 25). Thus the vertically oriented, hollow, cylindrical base of each leaf surrounds and incloses within itself the younger leaves and apical meristem. At maturity this cylindrical base of the leaf is the sheath or bulb-scale while the one elongated side is the blade. The rather angular shape of the younger leaves is determined by the surrounding structures, which limit and in a way mold the younger leaves within. During the differentiation into sheath and blade there is much rapid increase in the diameter of the growing region, so that the base of the newly formed leaf is pushed farther and farther away from the center (fig. 27). At the same time the thickness of the leaf blade and of the sheath continues to increase, so that by the time the next younger leaf is differentiated no more periclinal walls are formed. Further increase in the thickness of the sheath to form the fleshy bulb-scale is due to the formation and enlargement of intercellular spaces and to the growth of the cells.

The opening at the upper end of the sheath is designated, for convenience, the orifice of the sheath. A thin membrane extends around the upper edge of the orifice even on the side bearing the blade. Here the membrane is formed by an outgrowth, five or six cells thick, from the edge of the blade, and is usually without vascular tissue (figs. 27, 29).

In the first six or eight leaves elongation is proportionately greater than in later formed ones, and thus this orifice has become longitudinally much stretched so that its sides touch each other and close the cavity within until the next younger leaf is pushed through. In older leaves the tip of the blade is pushed through the orifice while both are inclosed within the sheath of the next older leaf.

In the formation of the first few leaves of a seedling, the blade attains a considerable length before the sheath begins to elongate appreciably. In a newly emerging leaf elongation takes place at about equal rates in all parts. The leaf tip is the first part to cease elongation, however, while the base of the sheath remains meristematic



FIGS. 17–26.—Fig. 17, longisection through stage shown in fig. 18; *a* and *d* are opposite sides of same leaf overgrowing the center (*bec*). Fig. 18, earliest stage of leaf differentiation. Fig. 19, cell detail of region *E* of figs. 17 and 25; plane of cell divisions marks boundary between regions *e* and *c*. Figs. 20, 21, one side (*c*) has outgrown the axis (*e*) and other side (*b*) and is beginning to overgrow it. Figs. 22, 23, both sides (*b* and *c*) overgrowing axis (*e*) which is itself beginning to extend upward. Corresponding parts have the same letters. Figs. 24, 25, the more rapidly growing side (*c*) has developed to form blade of leaf, whereas the upwardly growing periphery forms the sheath or bulb scale. (Small letters of first seven figures correspond to capitals in fig. 25.) *A* and *D*, *C* and *B*, and *a* and *d* are respectively the blade and the sheath sides of successively younger leaves (*p*, pith; *t*, tip of younger leaf). Fig. 26, arrangement of vascular bundles in mature leaf in region of leaf orifice (*a*, adaxial side of blade; *b*, branches from larger bundles; *c*, cross connections between adjacent vascular bundles; *e*, edge of orifice; *m*, bundle continuous in sheath and blade; *s*, outer side of sheath; *v*, bundles ending blindly under orifice).

longer than any other part. Thus in the earliest stage of development the entire leaf is meristematic; at a later date, only the sheath and base of the blade; and still later, only the base of the sheath. The lowest portion of a half-grown blade remains meristematic until the leaf is nearly grown, however, so that this region gives rise to a large share of the blade. Any one leaf, after it reaches a length of 3 cm., attains its mature length in a few days.

Mitosis continues in all parts of the leaf long after intercellular spaces appear (leaf *X* and younger, fig. 27). Transverse divisions as well as growth of the surrounding parenchymatous cells cause these spaces to become enlarged and elongated throughout the entire leaf. At the periphery of the blade in a very early stage of development there is differentiated what appears to be a meristematic layer (fig. 27). As the leaf elongates the cells of this layer do not become elongated longitudinally but radially, so that in the mature leaf they form two or three subepidermal layers of columnar cells corresponding to the palisade cells of dicotyledonous leaves. As these cells divide anticlinally they also increase the diameter of the blade. The inner parenchyma does not keep pace with increase in size, and so a huge central cavity which runs the entire length of the blade is torn through this inner spongy tissue. Before this cavity is formed all the inner cells of the leaf contain functional nuclei, although the volume of the intercellular space is probably greater than that occupied by the cells. After the cavity is torn the cells near its edge soon die, and so by their own collapse, according to NEWCOMBE (7), add to the size of the space. The mature leaf therefore consists of this central cavity, surrounded by eight or ten layers of parenchyma with large intercellular spaces, the vascular bundles, the palisade layers, and the epidermis.

Anatomy of leaf

Differentiation of leaf traces begins in the stem adjacent to its xylem, and proceeds upward into the developing leaf. In leaf blades which are no longer than they are thick (fourth younger leaf than *X*, fig. 27), procambial strands are already differentiated. Maturation of the bundles is endarch, beginning in the stem and, once begun in the leaf, proceeding upward rapidly to the tip. The first protoxy-

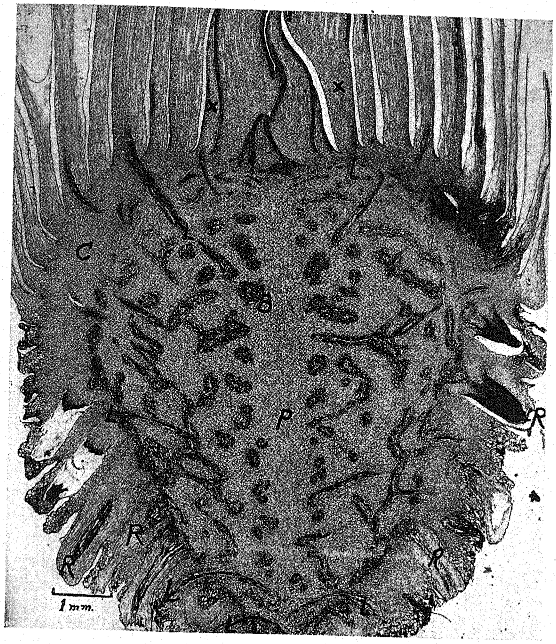
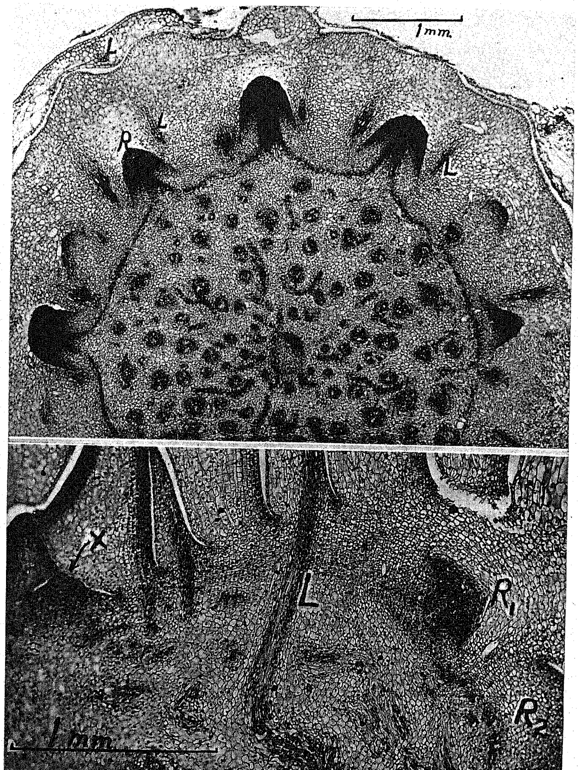


FIG. 27.—Median longisection, except at bottom, through plane of phyllotaxy of stem five months old; bulb development scanty, leaves 40 cm. long, and apical meristem at stage shown in fig. 21. Youngest five leaves contain mitotic figures. Row of dots in outer part of leaf X and of next older leaf are transections of connections between parallel leaf bundles. Darker tissue at edges of blades will become palisade tissue (B, transection through perimedullary bundle from which bundles of leaf arise directly; C, cortex; P, pith; L, vascular connections of leaf with stem; R, same of root with stem; X, sheath and blade of one leaf).



FIGS. 28, 29.—Fig. 28 (above), transection of stem five months old 50μ below apical meristem. Procambial strands of four adventitious roots (R) are continuous with outer procambial tissue of stem. Plane of phyllotaxy is parallel to side of page. Bundles shown in transection are arranged in seven concentric circles or arcs, one for each leaf. Of the innermost leaves only bundles of blade are shown, bundles of sheath not yet being differentiated. Radiating procambial strands inside of stele connect vascular or procambial tissue of developing roots and of leaves (L, vascular bundles of leaf). Fig. 29 (below), longisection through apical meristem and roots (L, vascular bundle of sheath of leaf; R_1, root primordium; R_2, older root; X, apical meristem).

lem is matured throughout the entire length of the leaf before any metaxylem is differentiated. Practically all of the xylem consists of spiral elements.

The cotyledon has only one vascular bundle; the second leaf has five; the next has usually eight; and successively older leaves may have one or two more bundles than the preceding leaf, until in the sheath of a mature bulb there are often as many as forty. These bundles are arranged around the periphery, run parallel throughout its entire length, and usually retain their individuality in spite of rather frequent small branchings and cross connections. These cross connections originate in one or several rows of parenchymatous cells which lie adjacent to and between the bundles. The cells divide at various angles periclinally and transversely to form many smaller cells which mature as a cross connection (fig. 27).

In both sheath and blade smaller and larger bundles may originate blindly or their bases may be anastomosed with larger bundles. Some of the smaller ones end blindly near the base of the blade. In the outer sheaths of a mature bulb there are very few cross connections between adjacent vascular bundles in the lower half, but many in the upper. There are cross connections at all levels in the unthickened sheath of a young onion. In outer sheaths of bulbs the vascular bundles often appear green because here a layer of subepidermal chlorenchyma almost surrounds them.

In a mature leaf the circular orifice of a young sheath has become oval and appears laterally placed, owing to a greater elongation of the sheath on the side bearing the blade. Near the base of the sheath vascular bundles are rather uniformly distributed, but near its top the bundles are more closely crowded together on the side from which the blade develops than on the opposite side. In the middle of this latter side are one or two smaller bundles which, after several branchings and cross connections, end blindly under or around the upper margin of the sheath. On each side of these is a larger bundle which parallels them up to the orifice and then is curved so as to parallel its edge. At the base of the blade these two bundles are again vertically oriented, making a sharp angle with their former course around the edge of the orifice. From this angle a smaller branch is continued around the periphery of the orifice

until, near the middle of the base of the blade, it meets a like branch from the other side. At the junction of these two a vertical bundle extends upward into the blade. Between this vertical bundle and each of the original large bundles is usually one other bundle extending upward from the branch around the top of the orifice. Frequently also the original large bundles have a vertical branch extending into the blade before they themselves become vertically reoriented. On either side of these two main bundles, the other bundles of the sheath are crowded together more closely near the sides of the orifice, but again acquire a uniform peripheral distribution in the blade (fig. 26).

In the cotyledon and in all subsequent leaves there occur longitudinal rows of elongated cells situated usually two cell layers beneath the epidermis. These are rows of lactiferous cells which are filled with a whitish fluid containing, according to RENDLE (9), resins and a substance easily hydrolyzed to allyl sulphide. It is this milky liquid which gives the broken onion tissue its characteristic taste and smell. The lactiferous cells in a longisection of a cotyledon 14 days old are easily recognizable, since they stain differently from the surrounding cells, are several times longer, and contain proportionately longer nuclei. In older leaves they occur just within the chlorenchymatous layers also. Their position has no relation to that of the vascular bundles. RENDLE believes that they probably perform either an excretory or a protective function, or both.

Stomata are numerous on the cotyledon of a seedling less than a week old. An epidermal cell and the two guard cells at one end of it were originally one cell. In the development of the stoma, this one cell, instead of dividing near its center as previously, divides at one end, cutting off a characteristically long epidermal cell and a square cell. This cell by one longitudinal division forms two cells which, as soon as the stoma between them is formed, become its guard cells. In a mature leaf the guard cells are sunken beneath the remainder of the leaf surface by more than their own thickness and the adjacent epidermal cells have overgrown them. The cuticle on the exposed epidermal cells is half as thick as the diameter of their own lumen. The surface of the leaf is glabrous and slightly glaucous.

Bulb

The onion bulb consists of a very short stem bearing adventitious roots and a series of thickened leaves and leaf sheaths, the older surrounding the younger, which have at their center still younger leaves and usually several apical meristems. The blades of the leaves may be functional, dried down, or, in the older of the inner leaves, undeveloped. In a large mature bulb the functional blades of the outer six or seven leaves have become dried and broken off, leaving only the sheaths. The outer of these sheaths have themselves also become dried and form a protection for the fleshy storage leaves within. Inside are three or four leaves with very short undeveloped but usually green blades, and inside these are successively younger leaves with blades proportionately longer as the leaves are younger and smaller. In the spring it is these youngest leaves that continue development and become green. The storage leaves outside of them also elongate, especially their sheaths, but their green blades remain relatively small. As the inner and younger leaves grow, the outer fleshy storage leaves become progressively dry from the top of the sheath to its base, because of withdrawal of stored food and moisture. Before leaf growth occurs, however, numerous roots are developed from the periphery of the stem. These extend into the soil and absorb moisture for this whole process.

The time at which formation of the bulb may be initiated varies. A bulb 1 cm. in diameter may be formed when only the fourth leaf shows, or eight or ten leaves may be formed before there is any evidence of thickening of the sheaths. In the first case the food which is being manufactured is stored, while in the second it is used in the formation of more and larger leaves. These two extremes may occur in plants growing under almost identical conditions.

Summary

1. The structure of the embryo and development of the seedling of *Allium cepa* are described.

2. The primary root is diarch. In its growing point are two histogens, the inner giving rise to the stele and the outer to the remainder of the root.

3. The adventitious root is usually pentarch, originates in the pericyclic region of the stem at the level of the apical meristem, and develops in a manner similar to that of the primary root. Secondary roots may be developed from the adventitious roots.

4. The first vascular tissue to mature is in the cotyledon. Xylem matures centripetally throughout this structure.

5. Transition from an exarch to an endarch condition of the xylem in the lower part of the stem is described.

6. The stem consists of a parenchymatous cortex, a stele which contains branched and anastomosed amphivasal vascular bundles in a groundwork of parenchyma, and a central pith surrounded by a cylindrical network of bundles to which most of the leaf traces are connected.

7. The development of the leaf is described. Vascular bundles of the leaf parallel each other, connect at frequent intervals, and are situated near the periphery of the leaf. The formation of guard cells and stomata is described.

8. Longitudinal rows of lactiferous cells are near the surface of the leaf but without relation to the vascular bundles. These rows never form vessels.

9. The structure of an onion bulb is described.

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CHLOROPHYLL CONTENT OF GRAIN SORGHUMS

J. C. Ireland and Parks A. Yeats

(WITH PLATE VII AND THREE FIGURES)

Introduction

In conducting an inheritance study in sorghum hybrids, it was observed that several selections showed pronounced chlorophyll deficiencies after four seasons of self-fertilization. Similar variations have been described by Conner and Karper (1), and our chlorophyll deficiencies seem to be included in their classification. There was some question as to whether the selections showing these deficiencies during the seedling stage would show any pronounced differences in yield or in later development.

The plan has been to make quantitative estimations of the chlorophyll at regular intervals during the growing season. A multiple correlation study of the parts of kafir plants was started about four years ago, and it was decided to include the chlorophyll content as a possible factor contributing to yields. Soil temperature, light intensity, and evaporation records have been made as a part of the experiment station routine. The correlation studies were of some value in determining the importance of physiological factors involved in chlorophyll deficiencies.

A study was made of the daily and seasonal changes in the chlorophyll content of grasses in South Africa by Henrici (4). She showed that there was a pronounced difference between the quantity present in the morning and that in the afternoon. This daily variation was influenced by the amount of moisture present. Following a heavy rain there was an increase in chlorophyll content, and continued drought caused a decrease in the content. High temperatures at night also caused an increase. Her estimations were made twice daily by extracting with acetone and separating with ether. Comparisons were made with a standard copper chlorophyll obtained from Stoll to determine the amount.

A later study of seedlings has been made, in which the plan of

LUBIMENKO and HUBBENET (6) has been followed. The microspectro-colorimeter replaced the photoelectric-colorimeter in order to make more direct comparisons and to eliminate errors. Decomposition of the chlorophyll and fatigue of the photoelectric cell were the most noticeable sources of error.

Methods

PLANT MATERIALS.—The variety of kafir employed was a cross of Blackhull White with Japonica. The plants were arranged in 22 rows, and were grown from selfed seed which had been carried for four generations for a multiple correlation study. The work was begun during the last week of June, and samples were taken from the rows each week of the growing season, until the grain was ripe and harvested, at the end of August. Leaves were pulled from the stalks at random and placed in a paper bag bearing the number of the row, collections being taken between 9 and 10 A.M. Two-gm. samples were clipped with scissors from each leaf, weighed immediately, and placed in extraction cups. Ten units of the Underwriters' Laboratories improved form of extraction apparatus were used to make the extraction, and 70 cc. of C.P. acetone was used in each flask. The operation was continued for three hours, and practically all the color was removed from the leaf particles, the acetone in the siphon cups being clear.

CHLOROPHYLL ESTIMATION.—A photoelectric-colorimeter was used to make a comparative estimate of the density of the extract. The glass container was filled with acetone extract and placed in front of the photoelectric cell window, with the galvanometer set so that the reading was zero. The amount of light reduction was recorded directly on the dial of the galvanometer. A second reading was taken by placing a standard green filter inside the lamp house and adjusting the pointer to zero on the dial. These readings were recorded to indicate the difference between a standard green and the acetone extract. This precaution was taken because it is probable that acetone extracted more than pure chlorophyll, and it is possible that the response of the photoelectric cell was not uniform for the different intensities of the medium. The standard solution as prepared by GUTHRIE (2) was used in estimating the amount. His prep-

aration is stable and corresponded favorably to the copper chlorophyll standard. He estimated that it was equivalent to 85 mg. of chlorophyll in a liter of water. In this manner it was possible to estimate the proportional part represented in our extract for the number of milligrams of chlorophyll present in 1 gm. of fresh kafir leaf. Since all of our samples received a uniform treatment, the results are comparable.

Eight additional rows of kafir were used for a second series of determinations. These rows were grown from seed which had shown chlorophyll deficiencies for four generations:

Row no.
145....Black Amber×Kaoliang
147....Black Amber×Durra (showing a 3:1 segregation of green and white seedlings)
150....Black Amber×Durra (showing a 1:1 segregation of green and virescent seedlings)
151....Black Amber×Durra
152....Black Amber×Durra
153....Black Amber×Durra
158....Reed's×Dwarf Br. Kaferita
159....Reed's×Dwarf Br. Kaferita

Duplicate plantings were made in small flower pots, one set being placed in a large earthenware jar and covered with black building paper to insure complete darkness; the others were placed in a well lighted part of the greenhouse. Temperatures of both groups of seedlings were as nearly equal as they could be maintained. After ten days, leaf tips were taken from seedlings, dipped in boiling water, and mounted in water and glycerin for examination with the microspectroscope. Those from the dark chamber were killed before being exposed to light in order to prevent the formation of chlorophyll.

A Zeiss microspectroscope (fig. 1) was used for comparing the leaf colors with a standard solution recommended by GUTHRIE (2) and photographs of the spectra were made for comparisons. (DuPont's 35 mm. panchromatic negative film was used in a Leica camera.) A low-power objective was employed for this examination, the lamps

being adjusted so that the spectra were of equal intensity before the solution and the leaf mounts were placed.

The Zeiss microscope is shown equipped with the tube attachments for solutions, the Zeiss microspectroscope in position, and a Leica film camera for photographing. The latter is supported upon an improvised crane with a tripod head attached to a large burette clamp. The upright is a rachet which may be raised or lowered with-

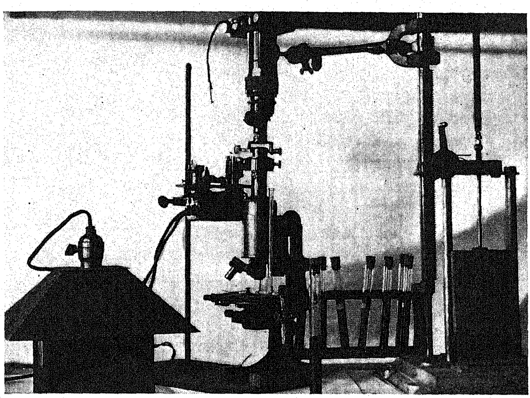

FIG. 1.—Apparatus for microspectral analysis; explanation in text

out throwing the camera out of alignment. In this manner the focus of the microscope may be adjusted and the spectrum observed without delay, and the camera may be quickly swung into place. A bulb control is attached to eliminate any vibration while the shutter is open. The lamps are upon a separate ring stand so that they may be adjusted without delay. Two 6–8-volt bulbs are mounted upon a bell-ringing transformer to provide light for the scale and the standard solution. The covered lamp was used for light through the microscope in order to eliminate any unnecessary light excepting that upon the mirror. The small bulbs are covered with caps when in use

so that their lights shine only upon the openings of the microspectroscope.

Seedlings were allowed to grow for ten days after the examination of leaf tips. Half-gram samples were weighed quickly, ground with washed sand, and treated with 20 cc. of 95 per cent alcohol until the chlorophyll was dissolved. The green extract was filtered into test-tubes. Eight cc. samples were pipetted into the colorimeter tube (fig. 1), the depth of solution in the tube being 8 cm. The apparatus was made according to the directions given by LUBIMENKO and HUBBENET (6). A uniform depth for each sample was easily obtained by filling the tubes to the same point upon the scale. Photographs were made of the spectra to show the comparative values of each band (pl. VII). A comparison was made with GUTHRIE's standard in the small vial attached to the side of the spectroscope.

DATA FOR MULTIPLE CORRELATIONS.—Charts were printed for keeping records of sorghum inheritance studies. Measurements had been made for four seasons of the several characters of the plants, which were considered as indicators of yield. One chart was used for the plants in a single row, averages being taken for each row measurement. The average length of seed branches was taken with a vernier caliper. The lengths of the plants were measured with a surveyor's rod, in feet and tenths of feet. The length of the head and the length of the internode were also noted at the same time. Circumferences of the heads and stalks were measured with a caliper. Counts were made of the number of nodes in the head, the number of side branches, and the number of leaves. The leaf area was estimated by circumscribing the leaf with a Dietzgen planimeter and calculating the area in square inches. This estimation was included in the correlation for 1932. It was used because of the general opinion that the greater the leaf surface, the greater the metabolism and consequently the greater the yield. The galvanometer readings for chlorophyll content represented a convenient range and were incorporated in the correlation of this year. The object of the entire correlation has been to determine mathematically what each character contributed to the yield.

TEMPERATURE AND EVAPORATION.—Records have been kept of soil and air temperatures, together with evaporation readings for the

experiment station. These were considered important in making a study of variations of the chlorophyll content of the leaves. Recording soil and air thermographs were used in making the weekly records of temperature. These were averaged with the planimeter, and the resulting figure was used as a value for that week. Readings of evaporation were made daily with a standard Weather Bureau

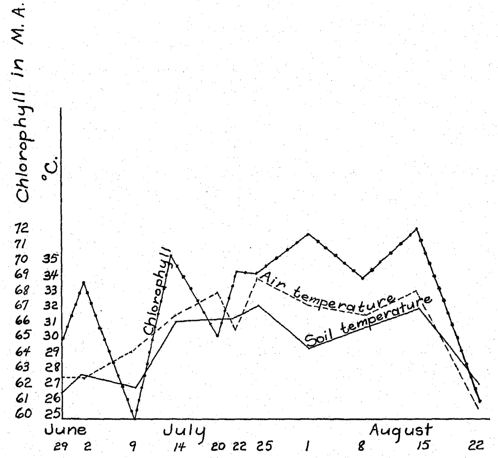

FIG. 2.—Comparison of chlorophyll content with air and soil temperatures

evaporation hook-gage. The averages of these were determined on the days when the chlorophyll content was calculated, so that each reading represents the approximate value for the corresponding periods. Soil temperatures were determined in a plot adjacent to the one in which the kafir was growing, the bulb having been placed vertically, below the surface of the soil. The Centigrade chart (fig. 2) represents a fair estimation of the temperature of the first 18 inches of soil.

Results

Chlorophyll extractions.—Table I gives the detail of galvanometer readings for acetone extracts made during the summer of 1932. There is a noticeable variation in amounts, those of the first day ranging from 56 m.a. to 72 m.a. Records show that most of the rows having a consistently low chlorophyll content had virescent or albino seedlings during the early stages of development. In the rows showing higher averages, the plants had a decidedly darker green foliage and were more vigorous. Plants of the tallest rows (Japonica) do not have the highest chlorophyll content but rather the lowest.

Reading vertically in table I, it may be noted that there is a pronounced irregularity in the determinations for the several periods. Henrici (4) shows similar deviations, and explains them as due to differences in moisture and temperature. If the differences were due to sudden changes in moisture supply, it would appear that all readings for that date should be low or high in proportion (note the results for August 12). The range is from 23 to 74, with about an equal number of high and low readings. Since the extractions were all made under uniform conditions, it is not probable that errors would cause such a difference.

The milligram weight of chlorophyll shown below the row averages in table I is the estimation based upon the standard used by Guthrie (2), in which he states that his solution is equivalent to 85 mg. per liter of water. Since our work was for comparative amounts, for correlation purposes, this estimation is offered as a basis for other work.

Figures 2 and 3 show graphically the trend of the chlorophyll content of leaves as compared with temperatures of soil and air, as well as with evaporation. There is a similarity of these trends, the chlorophyll variations apparently being less pronounced than the evaporation.

A check of experiment station weather records was made to determine whether these increases in chlorophyll were in any way correlated with cloudy or clear weather. There were only four cloudy periods during the entire time, as follows: the last two days in June; the 5th, 6th, and 7th of July; the 27th, 28th, and 29th of July; and the 15th, 16th, and 17th of August. The only observation that might be considered is that clear days following cloudy periods were

TABLE I

PHOTOELECTRIC-COLORIMETER READINGS IN MICROAMPERES OF ACETONE CHLOROPHYLL EXTRACTIONS

Date (1932)	Row numbers, Blackhull White × Japonica																						Average
	195	196	197	198	199	200	201	202	203	204	205	206	208	209	210	211	212	213	214	215	216	217	
June 29	64	63	60	65	61	61	64	67	63	72	62	71	76	44	75	70	68	56	66	67	64	72	65.0
July 2	65	70	68	65	70	69	71	72	69	70	....	....	....	....	....	....	....	....	....	....	....	....	69.0
July 9	58	59	60	52	67	55	68	65	68	60	58	58	54	54	62	67	60	58	50	67	54	64	60.0
July 14	68	67	62	60	77	70	79	76	73	74	....	....	....	....	....	....	....	....	....	....	....	....	70.6
July 20	65	61	58	52	67	61	68	55	54	67	65	66	71	71	57	69	75	70	62	72	72	77	65.2
July 22	77	75	68	60	69	67	69	70	66	70	70	68	73	73	72	72	70	67	67	73	74	61	69.6
July 25	73	72	74	70	61	67	75	70	67	74	69	73	73	68	71	65	73	64	65	70	71	70	69.8
Aug. 1	62	74	75	74	74	72	74	74	76	74	69	67	72	72	68	72	72	73	67	77	76	75	72.2
Aug. 8	69	69	73	68	71	71	72	70	72	73	65	52	64	69	68	66	70	68	63	78	82	77	69.5
Aug. 15	68	75	68	62	80	73	78	76	74	77	73	....	71	74	72	75	77	69	72	76	65	70	72.6
Aug. 22	23	67	37	51	66	60	68	67	46	74	62	....	....	68	79	74	74	46	64	68	62	58	60.7
Average	63	68.4	64.8	67.2	69.4	66	71.4	69.3	66.2	71.3	65.8	65	69.2	65.9	69.3	70	71	63.4	64	72	68.9	69.3	
Mg. of chlorophyll in 1 gm. of green leaves	3.09	3.34	3.18	3.28	3.4	3.2	3.5	3.4	3.2	3.5	3.2	3.2	3.4	3.2	3.4	3.43	3.48	3.1	3.13	3.5	3.37	3.4	

generally accompanied by increases in the chlorophyll content. Sunlight records, made by a Moll thermopile and recording galvanometer, did not show any pronounced variation in intensity, excepting during the cloudy weather. After the first of August there was a slight decrease in the intensity, but this was gradual and would not induce any pronounced variations in the chlorophyll content of leaves.

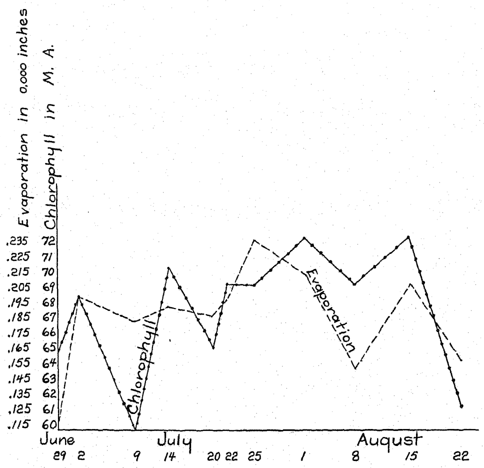

FIG. 3.—Comparison of chlorophyll content with evaporation

The drop in the curve at the close of the period indicates that the plants had reached a stage of maturity and that chlorophyll was not being formed. Practically all the leaves were dead, and the heads were harvested.

MULTIPLE CORRELATION.—The plan of WALLACE and SNEDECOR (7) was followed in the tabulation and solution of the multiple correlation. The array of figures was arranged on ledger paper for convenience in checking the results. In the first division, at the top of the page, the measurements and counts for 22 rows were listed.

The most interesting part of the correlation is the solution of the normal equation (table II). The correlation coefficients are listed in this so that a check may be made of the influences of the independent variables upon the yield. Starting at the bottom of table II, it may be noted that the yield correlates with the chlorophyll content to the extent of 0.535. Compared with the leaf area, the yield has a correlation coefficient of 0.456. The third value from the bottom is also interesting because it shows that the length of the last internode has a −0.572 correlation with the yield, indicating that the higher yields are associated generally with shorter last internodes. Continuing on up to the fourth item, the circumference of the head, it may be seen that it has a 0.859 correlation with the yield. Again the length of the head is negative, as shown in the third row of figures.

The score in percentages at the extreme right of the table is a solution of the Beta values of the correlation. The fractional part that each of these is of the total, gives this percentage. It is a comparative value, but is based upon the entire calculation. The high score of the circumference of the head suggests that it is the most important factor in determining yields. The chlorophyll content is next in value, with 22.94 per cent, and the leaf area is last, with 0.4 per cent. This percentage might be expected to be more, but it has been observed that many small leaved plants produce the greatest amount of grain.

The multiple correlation coefficient of 96.2 per cent is high, compared with those of previous years, and the standard error of estimate is low:

COMPARISON OF MULTIPLE CORRELATIONS (PERCENTAGE)

	1932	1931	1930	1929
Coefficients	96.2	76.2	89.0	74.2
Standard error of estimate	29.0	54.6	64.8	41.6

These comparative figures indicate that the 1932 estimations are considerably more accurate in determining the factors indicating yields. The standard error of estimate of 29 per cent shows that

TABLE II

SUMMARY OF 1932 CORRELATION COEFFICIENT

No.	Variables: 1. Average length of seed branch	2. Height of plant	3. Length of head	4. Circumference of head	5. No. of side branches	6. No. of leaves	7. Length of last internode	8. Leaf area	9. Chlorophyll concentration	10. Yield, the dependent variable	Score: Percentage contributed by each independent variable to yield
1.	1.00	−0.288	0.663	−0.214	0.070	0.016	0.227	0.468	0.281	−0.084	6.95 average length of seed branches
2.		1.00	−0.175	0.153	−0.392	0.371	−0.101	−0.002	−0.359	0.176	7.47 height of plant
3.			1.00	−0.607	−0.151	−0.133	0.347	0.243	0.172	−0.342	8.79 length of head
4.				1.00	0.448	0.263	−0.571	0.305	0.271	0.859	39.09 circumference of head
5.					1.00	0.149	−0.299	0.355	0.489	0.508	1.36 no. of side branches
6.						1.00	−0.117	0.563	−0.195	0.363	11.71 no. of leaves
7.							1.00	−0.297	−0.358	−0.572	1.29 length of last internode
8.								1.00	0.252	0.456	0.40 leaf area
9.									1.00	0.535	22.94 chlorophyll concentration
	Multiple correlation coefficient.............96.2%										100% yield
	Error of estimate........................29.0%										

there is still much to be desired in determining the yield values, but it is a decided improvement over the figures of 1930 and 1931. Table III shows the comparative scores over a 4-year period for the various measurements.

The fluctuations in some measured portions of the plant are of considerable interest. The relative value of the length of seed branches seems to be seasonal, with that in 1929 showing an unusually high score. Environmental variations rather than any trans-

TABLE III

SCORE OF RELATIVE WEIGHTS OF FACTORS INFLUENCING YIELDS

Factors	Percentage			
	1932	1931	1930	1929
1. Average length of seed branches	6.95	13.99	3.0	41.80
2. Length of plant	7.47	18.05	15.9	1.93
3. Length of head	8.79	16.03	13.3	11.93
4. Length of tip branches	0*	4.06	15.7	1.40
5. Circumference of head	39.09	24.52	26.4	24.44
6. Circumference of stalk	0	0.00	0.00	0.00
7. Number of nodes in head	0	1.05	7.8	2.87
8. Number of side branches	1.36	15.89	11.7	0
9. Length of last internode	1.29	6.41	6.2	15.63
10. Number of leaves	11.70	0	0	0
11. Leaf area	0.40	0	0	0
12. Chlorophyll content	22.94	0	0	0
Yields	100	100	100	100

* 0 indicates not determined.

mitted factors may be responsible for these changes. The length and circumference of the stalk have always been low. Because of these low scores, the circumference of the stalk, the number of nodes in the head, and the length of the tip branches were dropped from the 1932 calculation and were replaced by the number of leaves, leaf area, and chlorophyll content. The last is one of the most promising determinations. There may be some doubt as to the mathematical significance of the leaf area; however, physiological theory would naturally place the factor much higher than some of the others.

Spectral comparisons

Plate VII shows the comparative spectra of (1) leaf sections grown in darkness, (2) leaf sections grown in greenhouse light, and (3) al-

coholic solutions of chlorophyll from plants grown in the greenhouse for ten days after the first two samples had been taken.

It may be observed in the first column that there is some variation in the spectra of leaves grown in darkness. Spectrum no. 2 suggests the presence of some green coloring, and it may be due to the presence of chlorophyllogen, as suggested by LUBIMENKO. There seems to be a narrow band in the extreme red portions of the spectra at approximately 700 mμ. These bands in the red are also well marked in those leaves grown in light. Blue and green portions show different results in each selection. Spectrum no. 8 of those grown in daylight is of interest, because it is from leaves of the virescent type having almost no chlorophyll. The third column represents alcoholic solutions from various plantings of seedlings after they had grown to a height of 2–3 inches. No. 13 is a check spectrum of clear alcohol in the glass tube, shown in figure 1. Various widths of bands are observed above or below the 600 mμ mark on the scale. LUBIMENKO and HUBBENET (6) found the first bands in etiolated wheat seedlings at 670 mμ to about 620 mμ. Our seedlings were green, with the exception of those indicated "albino" (nos. 9 and 16).

The spectra of GUTHRIE's solution are shown at the right in each case. Those of the third column are much less pronounced because of a reduction in the time of exposure. The elimination of the portion of the spectrum below the lighter greens is of special interest. To the eye GUTHRIE's solution is identical with an alcoholic or an acetone solution. A photoelectric-colorimeter will not differentiate, but the microspectro-colorimeter shows a different type of selectivity. It is regretted that we did not have the crystallizable ethyl-chlorophyllide for use in a standard solution.

Determinations of the chlorophyll content of the kafir plants which produced the seed for these studies were made at intervals during the summer of 1932. The values were determined from the acetone extracted material, with the photoelectric-colorimeter.

Discussion

The use of the Underwriter's Laboratories improved form of extraction apparatus to obtain chlorophyll extractions made possible a uniform and rapid means of getting all the coloring matter from the

leaves of kafir employed in this investigation. Acetone may have dissolved other substances in addition to chlorophyll, but the number of samples taken and the extensive period of the project would hardly warrant a complete separation of all pigments.

The comparison of chlorophyll content with temperatures of soil and air and with the evaporation is in accord with the work of LUBIMENKO (5) and HENRICI (4). Higher temperatures suggest higher metabolic processes and consequently more chlorophyll.

TABLE IV

PHOTOELECTRIC-COLORIMETER READINGS

DATE (1932)	ROW NO.								
	145	147	150	151	152	153	158	159	AVERAGE
6/28	70	66	68	61	64	70	72	68	67.4
7/2	56	52	72	54	42	18	70	68	54.0
7/2	63	59	67	52	39	55	65	60	57.5
7/14	65	61	63	64	64	18	66	67	58.5
7/22	58	62	68	68	66	56	71	74	65.4
7/25	61	70	72	68	64	70	70	54	66.1
8/1	74	48	71	72	36	61	70	69	63.4
8/8	65	48	56	69	49	56	70	69	60.2
8/15	70	22	......	62	30	......	79	73	56.0
8/22	36	......	......	60	......	......	63	67	61.5
Average	61.8	54.2	69.5	63.0	50.6	50.6	69.6	67.4	
Mg. of chlorophyll in 1 gm. of leaf	3.03	2.65	3.41	3.09	2.47	2.47	3.41	3.30	

VON GUTTENBERG (3) also confirms this increase and decrease in his carbohydrate determinations in evergreens. The decrease in the amount of chlorophyll with the decrease in evaporation suggests HENRICI'S theory that chlorophyll contents change rapidly, even during the course of the day. The plastid being the basic structure, the chlorophyll seems to be deposited in the network as suggested by LUBIMENKO (5).

Summary

1. The chlorophyll content in kafir, based upon weekly determinations, increases generally to the stage of seed maturity in the plant. It begins to decrease as the grain hardens. The chlorophyll

content varies directly with the amount of evaporation and with the temperature.

2. There is a positive correlation between the chlorophyll content and the yield of kafir.

3. Varietal differences in the chlorophyll content seem to be consistent throughout the growing season.

4. The comparison of seasonal multiple correlation coefficients and their standard errors of estimate has made it possible to reduce the latter appreciably. The elimination of indefinite factors by the scoring process, and the addition of others, have aided in securing a higher correlation coefficient.

5. For comparative purposes, the microspectroscope is very effective in determining the relative concentrations of chlorophyll in kafir seedling leaves. These leaves are of uniform thickness when grown under the same conditions.

6. Solutions may be compared by the use of a microspectrocolorimeter with a greater degree of accuracy than with most colorimeters. Spectral band widths in alcoholic solutions vary according to the intensity of the chlorophyll.

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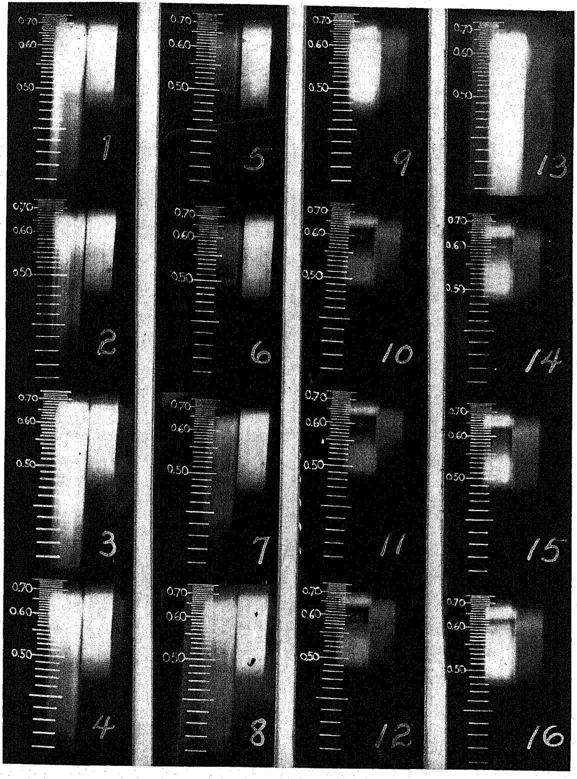

IRELAND & YEATS on SORGHUM

EXPLANATION OF PLATE VII

Comparison of spectra from kafir seedlings.

First column in darkness: 1, from row no. 145; 2, from row no. 147; 3, from row no. 150; 4, from row no. 151.

Second column in greenhouse light: 5, from row no. 145; 6, from row no. 147, green plants; 7, from row no. 147, albino seedlings; 8, from row no. 150, albino seedlings.

Third column, alcoholic solution of leaves: 9, from row no. 150, albino seedlings; 10, from row no. 151, green plants; 11, from row no. 152, green plants; 12, from row no. 153, green plants.

Fourth column, alcoholic solutions: 13, spectrum of alcohol, without chlorophyll; 14, from row no. 145, grown in darkness; 15, from row no. 145, grown in light; 16, from row no. 147, albino seedlings.

ROOT NODULE FORMATION ON THE GARDEN BEAN, STUDIED BY A TECHNIQUE OF TISSUE CULTURE[1]

KEITH H. LEWIS AND ELIZABETH McCOY

Introduction

Among the fundamental unsolved problems concerning the root nodules of the Leguminosae is the relation of the rhizobia and their hosts before and during the process of nodule formation. The factors which stimulate the bacteria to enter in the first place, the method of their entrance, their complex relation to host during nodule development, and the basis of their specificity for certain host plants (that is, the complete immunity of non-leguminous plants and the well known cross-inoculation grouping within the Leguminosae) are unknown. The interaction of plant and bacteria may indeed begin before the bacteria enter at all, for it is well known that plants exert a certain influence upon the general soil microflora (14, 6). It is assumed that the greater multiplication of bacteria in the rhizosphere of plants is due to the greater concentration of organic matter, either from direct root excretion or from the sloughing off of dead cells. WILSON (20) and JOSHI (7) have further indicated that leguminous plants are in some way particularly favorable to maintenance of the rhizobia in the soil surrounding their roots.

In an early report on the mechanism of infection, it was suggested that leguminous plants through some root excretion exert chemotactic attraction for the rhizobia of the surrounding soil. On the other hand, many have held that chance contact of rhizobia with the numerous root hairs is sufficient to account for infection (FRED, BALDWIN, and McCOY 5). THORNTON (15), however, has revived the earlier conception on the basis of his observation that the young alfalfa plant, at a stage of development coincident with the opening of the first true leaf, produces a substance which influences infection. Younger seedlings are seemingly immune to infection, although exposed to excessive numbers of active rhizobia. Infection, however,

[1] Published with the approval of the Director of the Wisconsin Agricultural Experiment Station.

may be induced in the young plants by the presence of older plants or of washings from soil about their roots. The stimulating substance therefore appears to be given off from the roots of plants of proper age and to be water-soluble. THORNTON tried to determine the source of the substance by cutting off various parts of the tops, and came to the conclusion that the removal of the cotyledons materially delays the formation of nodules, whereas the removal of the first true leaf or terminal bud has practically no effect. He did not definitely prove the source or nature of the material, therefore, but rather suggested that some general physiological change occurs in the plant, causing liberation of the specific substance at the time of opening of the first true leaf.

That the plant exerts some control over infection is evident in other ways also. THORNTON (16) pointed out that there is a definite limitation of the degree of infection, only 4 per cent of the root hairs of alfalfa plants grown in agar being infected even in the presence of excessive numbers of rhizobia. FRED, BALDWIN, and McCOY (5) cited a case of secondary setting of nodules upon plants of *Phaseolus vulgaris*, from which the seed pods had been removed before maturity. And DUNHAM and BALDWIN (3) found in their experiments with double inoculation that nodule-free plants of a given age were much more readily infected with an effective strain of rhizobia than were parallel plants already bearing nodules of an ineffective strain.

The delicate balance between the rhizobia and their leguminous host is also evidenced by the effect upon the association produced by changing environment. The effects of the various environmental factors have long been studied, primarily as affecting the number, size, and placing of nodules, and effectiveness or resultant benefit to the host. In summary it may be said, ". . . . any factor which tends to promote vigorous plant growth, with the exception of a plentiful supply of combined nitrogen, will enhance the benefit derived by the host" (5). In general, the optima for effective nodulation are a plentiful supply of air, moist but not water-logged soil, adequate sunlight, a temperature favorable for plant growth (about 25° C.), neutral soil reaction, and a well balanced nutrient solution rich in the essential elements except nitrogen, which must be low for best setting and functioning of nodules.

Of these several factors only light need be discussed here, since it is the only factor purposely varied in the present work. The depressing effect of inadequate lighting was noted as early as 1888 by VINES (18), who reported that *Vicia faba* grown in shade produced no nodules. PRAZMOWSKI (9) in 1890 grew pea plants in garden soil for 4 weeks in a darkened room and found nodules as numerous upon them as upon control plants grown in light. They were definitely smaller on the darkened plants, however. Recently LEONARD (8) concluded, from a long series of greenhouse tests, that it is chiefly lack of light which causes poor nodulation and poor plant growth during the dark winter months. And since reduction of chlorophyll (by removal of leaves) or insufficient CO_2 supply produces a similar result, it appears that limitation of photosynthesis by any of these means may be responsible for poor nodulation. THORNTON (17) also linked the depressed nodulation of his darkened alfalfa plants with lack of photosynthesis. Upon plants kept in complete darkness from germination onward only 3.5 nodules per 20 plants were formed; upon those seedlings darkened after 12 days of growth in light (that is, at the age of opening of the first true leaf), 16 nodules per 20 plants; and upon control plants continuously in the light, 46.5 nodules per 20 plants. It was also noted that upon the second lot of plants, those nodules initiated during the period of light ceased growth after darkening, and that no new nodules were formed. In other words, there appeared to be a direct relation between photosynthesis and development of nodules. WILSON (21) demonstrated practically the same point in a different manner. By supplying carbohydrate in the form of dextrose, levulose, or sucrose in 0.5–2 per cent concentration in a mineral salts nutrient agar, he was able to grow plants in complete darkness at 25° C. for as long as 36 days. Under such conditions no nodules were ever formed on alfalfa or clover, but two nodules developed on a pea plant in the presence of 2 per cent dextrose. The vetch plants gave somewhat better results, one nodule being formed even on a control plant without sugar, two on a plant with 2 per cent dextrose, and as many as four on a plant with 0.5 per cent sucrose. Thus in spite of the large number of plants failing to form nodules, it would seem that in the presence of carbohydrate, suitable in kind and concentration, infection and at

least early stages of nodule formation may be induced upon entirely etiolated plants of the Leguminosae.

The experiments to be described in this report have been designed to study further aspects of the complex relation between bacteria and plant, suggested by the work of THORNTON and WILSON. They have therefore taken two directions: (1) the attempt to produce nodules upon excised roots to determine whether there is evidence of the stimulatory root excretion by such (if so, it would necessarily be a localized root product, entirely divorced from any photosynthetic process in the top); and (2) the extension of the WILSON technique to another plant, *Phaseolus vulgaris*, in a different cross-inoculation group. The bean seems a particularly hardy plant for laboratory experimentation and may therefore prove a better host for nodulation than the plants used previously.

Experimental work

The culture of excised roots (that is, of root systems from which the tops have been removed as soon as the roots have completely emerged from the seed coat) is not new. There is in fact a comparatively extensive literature of reports upon growth of dissected plant parts, dating back to 1754 according to a review by ANDRONESCU (**1**). The most notable recent work on root culture is that of ROBBINS (**10, 11**) and of ROBBINS and MANEVAL (**12, 13**). These papers describe a method whereby excised roots of various plants have been kept alive for as much as 12 weeks; they also discuss some of the factors, such as nutrition, light, and aeration, which affect the growth of excised roots.

Preliminary to the experiments of this report, five series of tests were run to explore the possibilities and to find suitable conditions for culture of the excised roots of the desired leguminous plant. Various plants, including clover, pea, alfalfa, lima bean, and wax bean, were tried. The last, locally known as Old's Pencil Pod black wax bean, a variety of *Phaseolus vulgaris* L., was finally chosen for most of the work; the hairy vetch, *Vicia villosa* Roth., was also used in checking these experiments with the result obtained by WILSON with whole etiolated plants. In this early work about 25 variations of four fundamental salt mixtures were used, including the use of

plant extract sterilized by Berkefeld filtration, omission and substitution or removal of nitrogenous compounds from the media, the use of agar, liquid, cellulose, glass bead substrates, etc. Commercial glucose (2–4 per cent) and 400 p.p.m. of autolyzed yeast were in every case added to the media. While no nodules were formed in any of these tests, it was repeatedly noted that the best growth of the roots was always associated with the presence of nitrate. And since this form of nitrogen is particularly depressing to nodulation, many of the variations tried were intended to replace it, but without success so far as nodulation was concerned.

The main experimental work was carried out in two series, the first of which was an attempt to overcome the difficulties encountered in the preliminary tests, and the second to confirm and broaden the suggestive results of the first series. Both were based upon the WILSON technique with modifications, which will be detailed for the first series only.

EXPERIMENT I

The seeds of black wax bean and vetch were sterilized by first washing in distilled water, immersing for 1 minute in 95 per cent alcohol, then for 10 minutes in 1:1000 mercuric chloride, and finally washing 4–5 times in sterile distilled water. The seeds were then allowed to germinate in the dark in petri dishes at 28° C. on yeast water mannitol agar (medium 79 of FRED and WAKSMAN 4). This nutrient agar was intended to favor bacterial and mold growth and thus to facilitate the detection of any non-sterile seeds.

The medium for culture of the plants was the regular WILSON formula:

$MgSO_4$	0.2 gm.
KH_2PO_4	0.2 "
NaCl	1.0 "
$CaSO_4$	0.1 "
Agar	15.0 "
$CaCO_3$	In excess
Water	1 liter

Heat the salt and agar mixture thoroughly to mix and dissolve, add the desired sugar (in this case 0.5 per cent

sucrose), and sterilize in suitable containers (in this case 32 oz. round glass bottles having cotton plugs and containing 250 cc. of the medium). Finally add sterile $CaCl_2$ solution to give a 1:500 molar concentration.

After two days' germination the seedlings were transferred aseptically to the culture bottles. In all, 24 bottles were used for this series, 12 planted to vetch and 12 to beans with 3–5 seedlings in each case. The bottles were then set in closed heavy paper boxes to keep out all light and were placed in a dark 28° C. incubator. The temperature varied actually from 24° to 29° but averaged about 27° C. After two days more of growth the roots were aseptically severed from the tops in seven bottles each of beans and vetch; the tops were not removed from the bottles. The 12 bottles containing beans were then inoculated with 1 cc. of a heavy suspension of *Rhizobium phaseoli* Dangeard, strain 405 of the University of Wisconsin collection, and the 12 bottles containing vetch were similarly inoculated with a suspension of *Rh. leguminosarum* Frank, strain 325. The bottles were immediately returned to the dark incubator and examined only occasionally with artificial light.

The cutting of the roots did not seriously injure either tops or roots, for after a few days the tops sent out strong adventitious roots from the periphery of the cut surfaces; they continued to grow as well as did the plants which were left intact. The excised roots also soon began to stretch over the surface of the agar, and upon reaching the side, turned down between agar and glass. As growth continued the roots branched and rebranched, forming numerous long root hairs which were often visible to the naked eye. In later stages of growth the excised vetch roots began to lag, failed to produce more secondary roots, and became brown and soft. The vetch roots attached to tops grew somewhat better, but they too appeared spindling and discolored and ceased to grow after reaching a length of 10–15 cm. The excised bean roots showed stronger growth, however, often reaching a length of 15 cm. or more and producing 30–50 secondary roots. They were not discolored and appeared to be morphologically normal. The bean roots attached to tops grew even more hardily, as judged by total elongation and number of long and rebranched secondary roots.

Other characters of growth were that all plants were very brittle and had little tensile strength in comparison with sturdy plants grown out of doors; that they were entirely etiolated; and that drying of the exposed roots was not a serious factor in the experiment, owing to the humidity of the incubator and the scheme of incubation in closed boxes.

Sixteen days after inoculation the first macroscopic nodule was observed on the root of a whole vetch plant, and on the 22nd day one on an excised bean root and three on bean roots attached to tops. On the 27th day all of the bottles which appeared to contain

TABLE I

NODULE FORMATION ON EXCISED ROOTS AND ETIOLATED PLANTS OF BEAN AND VETCH; EXPERIMENT I

	BEAN	VETCH
Total number of excised roots	28	28
Total number of plants with adventitious roots only	28	28
Total number of whole plants	20	20
Number of excised roots having macroscopic nodules	1	0
Number of plants with only adventitious roots having macroscopic nodules	2	0
Number of whole plants having macroscopic nodules	4	1
Total number of macroscopic nodules	9	1
Maximum number of nodules per root	2	1

nodulated plants were cut open with an electric glass cutter, the plants removed to pans of water, and their roots carefully washed free from agar. A summary of the nodulation found appears in table I. The small number of macroscopic nodules formed clearly indicates that optimum conditions for nodule formation had not been supplied for either etiolated plants or excised roots. The results, however, do demonstrate that whole etiolated plants of both bean and vetch will produce nodules under the conditions of this experiment; and further that adventitious roots produced upon the stem in absence of the original root system, as well as excised roots of the bean grown in the presence of the tops re-rooted, can also form nodules under these conditions. A detailed study was made of those plants bearing nodules, with the results shown in table II. The

TABLE II

DETAILED OBSERVATIONS ON EXCISED ROOTS AND ETIOLATED PLANTS OF BEAN AND VETCH; EXPERIMENT I

Plant	Number of nodules		Infected root hairs	Condition of root hairs	Condition of root	Length of root (cm.)
	Large	Microscopic				
BEAN						
Top with adventitious roots......	2	2	+	Very numerous; many curled at tip, bulbed, branched, some stubby	Tips normal; numerous side roots	13
Whole plant..	2	0	−	Numerous; straight, curled, and bulbed; some stubby, others collapsed	Slightly brown, tips normal, many side roots	21
Excised root..	1	1	−	Scarce, short, otherwise normal	Slightly brown, fewer side roots than above, tip of tap root somewhat swollen	9
Whole plant..	1	3	+	Numerous; many stubby, bulbed, curled, or branched	Slightly brown, many side roots	18
Whole plant..	1	2	+	Numerous; curled, some stubby, bulbed; ends rough	Slightly brown, numerous side roots, tips normal	15
Whole plant..	1	2	−	Numerous; long, bulbed, curled	Slightly brown, many side roots, tips normal	19
Top with adventitious root.......	1	1	+	Numerous; long, bulbed, curled	Slightly brown, many side roots, tips normal	19
VETCH						
Whole plant..	1	0	−	Numerous, long, collapsed	Dark brown, few side roots, brittle	

column headed "Number of nodules . . . . microscopic" refers to those enlargements of the roots that were not readily visible to the naked eye, but which under the low power of the microscope appeared to be very young nodules, distinguishable with fair certainty from young secondary roots. The column "Infected root hairs" is significant only for its positive entries; negative signs mean only that no infected hairs were found.

Free-hand sections of a number of the nodules were made; they were treated with very dilute methylene blue and examined wet under the 4 mm. objective. All of the sections observed appeared morphologically normal, having as the outstanding characters well developed vascular strands and many large cells in the bacteroid area packed with bacterial bodies. The only detail in which they were found to differ from published descriptions of normal bean nodules (5) was in the absence of stored starch. This is not surprising, since no starch was found by the iodine test in any part of the etiolated plants. Perhaps in the presence of a low concentration of sugar (0.5 per cent) there is no excess absorbed and stored. Another point of interest about the tissues of these nodules was the absence of any sign of that parasitism noted by THORNTON (17) in his darkened nodules. This fact is really indirect support for the hypothesis of THORNTON, that the attack of the bacteria upon the tissues of their host is induced by lack of carbohydrate food. At any rate the present work indicates that bean plants or parts of plants grown entirely in the dark but supplied with proper nutrient solution, lacking nitrogen but including carbohydrate, can produce normal nodules.

EXPERIMENT II

The data of experiment I are obviously insufficient for sound interpretation of results because of the small number of plants in the series, and because of the uncertainty whether the results could be duplicated with any reasonable regularity since the factors controlling nodulation under these artificial conditions are so poorly known. Also the plan of experiment I offered no check on the possibility of nodulation of excised roots alone in a bottle, the excised tops having re-rooted and grown with the excised roots in all cases. In other words, it was not proved in experiment I whether

the excised roots were infected because of the presence of that hypothetical substance originating in the tops, or whether they were self-sufficient in that respect. Experiment II was therefore set up as a repetition of experiment I, with certain additional lots to round out the series. The plan was briefly as follows:

3 whole plants per bottle	20	bottles
3 excised roots (tops removed) per bottle	20	"
3 excised roots plus tops with adventitious roots per bottle	20	"
2 excised roots (tops removed) plus 1 whole plant per bottle	20	"
3 whole plants per bottle[2]	20	"

The seeds, beans only, were sterilized, germinated, and planted as before. Because this series was carried out in midsummer, the temperature usually ranged between 28° and 30°, but at times went as low as 25° or as high as 33° C. In other words, it tended on the average 2° or 3° higher than in the earlier experiment.

Twenty-four days after inoculation the bottles were removed from the dark room and examined. No nodules were found on the 60 whole plants raised in absence of carbohydrate. This is somewhat surprising, because PRAZMOWSKI (9), THORNTON (17), and WILSON (21), whose work has been discussed in the introduction, each observed in rare cases the nodulation of an etiolated plant in the absence of added carbohydrate. It is known that plants vary in their physiological properties, of course, and it may be therefore that the black wax bean is unable to develop nodules without abundant sugar. The nodules in the remaining lots of plants were found upon the surface roots or those between agar and glass; better aeration to these roots or the difficulty of root penetration in 1.5 per cent agar may account for this tendency. Table III gives the results obtained. It appears from the data that the conclusions of experiment I are confirmed, that it is possible under the conditions of the experiment to induce nodulation of whole etiolated bean plants, tops with adventitious roots, or (more rarely) excised roots in the presence

[2] These whole plants were in WILSON's agar, sugar omitted, to determine whether the presence of carbohydrate was the controlling factor for infection.

of tops re-rooted. And it may be added from experiment II that it is possible, although still more rare, to obtain nodulation of excised

TABLE III

NODULE FORMATION ON EXCISED ROOTS AND ETIOLATED PLANTS OF WAX BEAN; EXPERIMENT II

	Excised roots	Top with adventitious roots	Whole plants
Total number used	160	60	80
Total number with nodules	6	11	14
Number grown alone	60	....	60
Number with nodules (grown alone)	1	....	9
Number grown together (excised roots+tops with adventitious roots)	60	60	....
Number with nodules (excised roots+tops with adventitious roots)	3	11	....
Number grown together (excised roots+whole plants)	40	....	20
Number with nodules (excised roots+whole plants)	2	....	5

bean roots entirely alone in a bottle. Perhaps these relations will be seen more clearly in a percentage evaluation of the data:

		Percentage of plants showing one or more nodules
Whole plants alone		15.0
Together	Whole plants	25.0
	Excised roots	5.0
Together	Tops with adventitious roots	18.5
	Excised roots	5.0
Excised roots alone		1.7

The maximum number of nodules on any one excised root system was four, on an adventitious root six, and on a whole plant ten. Thus while nodules can be induced to form on excised roots, either alone or in the presence of whole plants or of tops re-rooted, it cannot be claimed that they form as readily as upon whole plants or upon tops with adventitious roots. Also it is apparent, from the comparatively low percentage of whole plants forming nodules in these experiments, that the absence of light interferes with nodule development in some way which the addition of sugar does not entirely overcome.

As to the possible working of a root secretion stimulatory to infection, as proposed by THORNTON (15), there is little evidence one way or the other in the present experiment. The fact that nodules did form on an entirely isolated root (indeed four nodules, or more than on any other excised root in the experiment) is positive evidence that infection did occur without any possibility of that hypothetical substance originating in the top. But the case is 1 positive to 59 negative which had the same opportunity. The fact that excised roots in the presence of whole plants or of tops with adventitious roots did present slightly higher positive figures (two in 40 and three in 60 respectively) is perhaps significant, but again the figures involved are too small for conclusive results. Furthermore, the fact that nodules form less readily on excised roots than on roots attached to tops is not necessarily proof that normally the roots derive from the tops some substance beneficial to nodulation. The alternative explanation, that in the excised root there exists some unrecognized pathological condition, is quite as defensible. It is true that the excised root, as just stated, appears to be morphologically whole, but of its physiological normality we have no knowledge. Growth is our only criterion, and that is admittedly depressed under the conditions of these experiments.

Summary

1. Excised roots and etiolated plants of the black wax bean, a variety of *Phaseolus vulgaris* L., have been raised in artificial culture by an adaptation of the WILSON technique. Growth of the roots is admittedly not normal, in amount at least, as compared with the standard for root development of field plants. (WEAVER and BRUNER [19] state with reference to beans: "Plants only in the cotyledon stage, but grown in warm, mellow soil, often have well-branched tap roots twelve inches long.") In the present experiments the roots usually attained about half that length, occasionally two-thirds. They were also more brittle than normal but no regular morphological abnormality could be found.

2. Nodulation (*a*) of excised roots (grown alone or in combination with etiolated whole plants or tops with adventitious roots), (*b*) of etiolated tops having adventitious roots only, and (*c*) of etiolated whole plants of the bean has been accomplished.

3. The ratio of nodulated roots of the various types to the corresponding non-nodulated is low, and indicates that some factor, as yet unknown, is depressing nodule development. It appears further that sugar is essential to nodulation in these experiments but does not entirely overcome the effect of absence of light. The excised roots are distinctly less susceptible to infection and development of nodules than are the roots of etiolated whole plants or of tops re-rooted.

4. The nodules studied were histologically normal but lacked the usual starch of the bean nodule. Possibly this lack indicates merely that no excess was absorbed and stored from the 0.5 per cent sucrose in the nutrient. There was no sign of that parasitism noted by THORNTON (17) and attributed to lack of carbohydrate food in his darkened nodules.

5. Study of the results of these experiments for their bearing on the question of a root secretion stimulatory to infection shows little either way. Nodulation of entirely isolated excised roots is positive proof of infection unaided by any hypothetical substance originating in the tops, as late as that reported by THORNTON at least. The increase in nodulation of excised roots grown with whole plants is slight and certainly insufficient to indicate the working of a root secretion. The considerably increased nodulation of etiolated whole plants or tops re-rooted might suggest the presence of a substance derived from the tops and favorable to nodulation. But there is no guaranty of the normality of the excised root. Some pathological condition, depressing to nodulation, may account for the apparent difference between nodulation of the excised root and of the etiolated whole plant.

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SPORE FORMATION IN SCLERODERMA LYCOPERDOIDES

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(WITH NINETEEN FIGURES)

Introduction

Cytological studies of spore formation in the Gasteromycetes have been few. MAIRE (5) reported that, at the beginning of the prophases of the first nuclear division in the basidium of *Scleroderma vulgare*, the cytoplasm contains a certain number of granules, stained black in iron-alum haematoxylin, from which radiate the microsomes. Two of these granules become centrosomes, acting as the center of radiation. They then become located at opposite sides of the nucleus, whose nucleolus and membrane soon disappear, while the chromatic reticulum is transformed into two irregular knotty clubs which represent two chromosomes and extend almost from one centrosome to another. The spindle is organized between the centrosomes; the two chromosomes contract, then divide longitudinally, and two daughter chromosomes thus pass to each pole. At the pole a chromatic mass covers the centrosomes from which astral rays still radiate. The spindle soon disappears and the second division, which is like the first, begins almost immediately. There is formed around each nucleus a spindle with centrosomes and asters.

RUHLAND (8), PETRI (6), and BAMBEKE (1) agree that the basidium in *Hydnangium carneum* is binucleate at its origin. Fusion of the two nuclei occurs in the spireme stage. PETRI has pointed out that "granulation directrices" separate out from the nuclei and pass to the summit of the basidium. The first division spindle, with a centrosome at each pole, is apical and transverse to the longitudinal axis of the basidium. BAMBEKE found protochromosomes similar to those described by MAIRE, which are replaced by two definite chromosomes at the end of the prophase. The daughter nuclei divide simultaneously. The spindles of the second division are likewise transverse in the basidium. PETRI concluded that a true spindle is

not formed, and that the daughter nuclei are composed of groups of granules without true nucleoli, which are attached to fibers from the base of the sterigmata. According to RUHLAND and BAMBEKE, however, four nuclei with nucleoli and membranes are present at the base of the basidium after the conclusion of the second division. Granules and aster-like radiations are present at the points of origin of the sterigmata. After completion of the second division, the nuclei occupy a clear space in the apical portion of the basidium, become elongated, and each apparently is composed of a mass of granules. RUHLAND and BAMBEKE found that the entire nuclear material of one nucleus streams through the sterigma into the spore, and that a nuclear membrane is formed immediately in the spore. PETRI and ISTVANFFI (4) reported that only a part of the chromatic substance passes through the sterigma into the spore in the form of granules, and that the nucleus is organized from these granules in the spore. In some cases the spore is small while in other cases it is large at the time of passage of the nuclear material. RUHLAND found that one, two, or even three nuclei may pass to one spore, but BAMBEKE reported only one nucleus to a spore. This nucleus later divides.

FRIES (3) found the fusion of the two nuclei in the spireme stage in the basidium of *Nidularia*. Vegetative nuclei measure 1–1.5μ, while the fusion nucleus which practically fills the swollen basidial tip is 5μ in diameter. The fusion nucleus passes through synizesis and later forms a spireme. Longitudinal splitting, segmentation, and contraction of the chromosomes follow. In the prophases the membrane disappears. A long narrow spindle placed transversely to the longitudinal axis of the basidium is formed. Centrosomes are present at the poles. In the anaphase of the first nuclear division, FRIES saw three or four chromosomes at each pole; but at the conclusion of the second division only two chromosomes appear there. After the first division, without reorganization of the nuclei new spindles are formed in an oblique, transverse, or perpendicular position. After the second division, four resting nuclei with membranes are present at the base of the basidium. Granules were seen in the basidium at the base of the sterigmata, and cytoplasmic strands seemed to stream from them to the nuclei. As the spores are formed the nuclei appear to have lost their membranes and to have become

elongated. The chromatic material becomes attenuated and streams through the sterigmata. The nucleus of each spore divides, the mature spores thus being binucleate.

Accounts of the formation of the fruiting bodies of *Scleroderma* and the development of the cavities containing basidia have been given by TULASNE (10), SOROKINE (9), and RABINOWITSCH (7). The young fruiting body consists of a solid undifferentiated mass of tangled, branching hyphae. Although TULASNE thought that various branches of these hyphae form basidia, RABINOWITSCH and SOROKINE found that all the basidia in a cavity arise from the branching of one hyphal knot. Lateral branches become basidia and the surrounding hyphae disappear. Hyphal knots and later basidial cavities appear first in the center of the cavity and later ones develop in a centrifugal order.

MAIRE found the number of spores in *Scleroderma* to be four; TULASNE and RABINOWITSCH found normally four but also variations from two to five; while SOROKINE occasionally found only one spore.

MATERIAL AND METHOD.—The material of *Scleroderma lycoperdoides* Schw. used in this investigation was found on a rotted log in a dense cedar swamp near Douglas Lake, Michigan. The fruiting bodies were dissected from the wood and fixed in formalin-acetic-alcohol and in Flemming's medium solution. Sections were cut at 5μ and stained with Haidenhain's iron-alum haematoxylin or with Flemming's triple stain.

Investigation

The basidia arise as lateral branches of a twisted intertangled hyphal knot as described by SOROKINE and RABINOWITSCH. They are first detected by their darker staining reaction and by their somewhat swollen tips (fig. 1). Basidia of one knot grow toward one point, the knot enlarges, and the hyphae and basidia spread farther apart. The basidia ultimately increase to five times their original size in diameter, and four spores are formed from each. The surrounding hyphae disintegrate, and when the spores are mature the basidia likewise disappear.

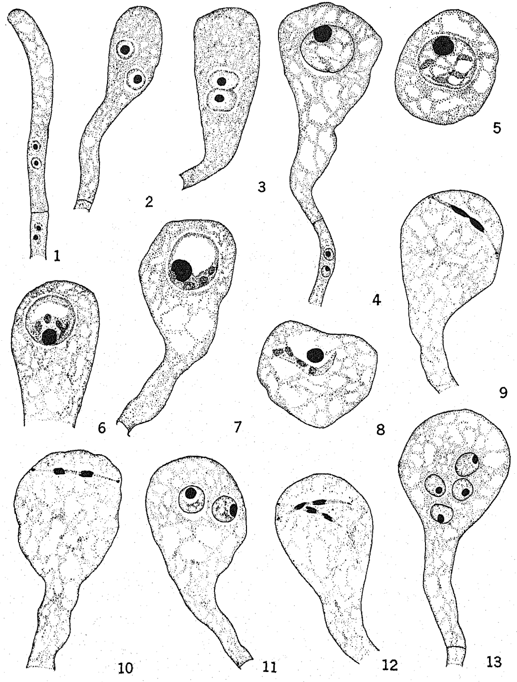
All the cells of the young fruiting body are binucleate. The cell which will become the basidium is 1.6μ in diameter when first recognized, and contains two nuclei which are usually close together.

These nuclei move toward each other and lie in close proximity for a short time. Then they come in contact, become flattened in the plane of contact (fig. 3), the membranes disintegrate, and complete fusion follows. At the time of fusion the basidium is 3.5–4 μ in diameter and the nuclei are approximately three times as large as when first observed.

The fusion nucleus, 2.5–3μ in diameter, is in the apical portion of the swollen basidium. It contains a large nucleolus, around which is a clear space, at least in fixed material. Throughout the rest of the nuclear cavity, however, is a chromatic reticulum in which more darkly stained portions may be distinguished (figs. 4, 5).

In preparation for the division of the fusion nucleus, the chromatic material and denser nucleoplasm become massed toward one side of the nucleus, surrounding the conspicuous nucleolus, which is often pressed against the nuclear membrane (fig. 6). This stage resembles synizesis, characteristic of the heterotypic prophases in higher plants. The synizetic mass tends to spread outward, tapering along the nuclear membrane (fig. 7). In the next stage found the membrane had disappeared, the densely staining mass retaining its shape. Later a long narrow spindle, lying in the apical portion transverse to the longitudinal axis of the basidium (fig. 9), is formed with the chromatic mass at the center. The spindle poles reach the walls of the basidium and at each pole there is a darkly stained body suggestive of a centrosome.

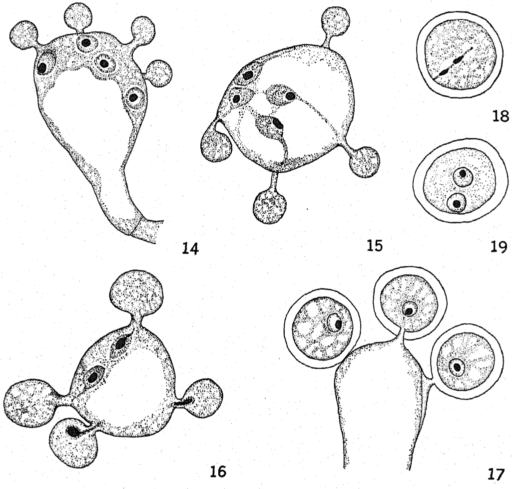
The chromatin mass divides and the halves separate and move toward the poles (fig. 9). In some cases two bodies suggestive of chromosomes may be distinguished in each daughter mass (fig. 10). Two daughter nuclei are organized (fig. 11). The rarity of the two-nucleate as compared with the one- and four-nucleate stages suggests that interkinesis is of short duration. The second division, occurring simultaneously in both daughter nuclei, takes place rapidly. The spindles of this division, like that of the first, lie in the apical portion of the basidium transverse to its longitudinal axis; they make a right or oblique angle with each other (fig. 12). The spindles are in such close proximity that it is often difficult to distinguish one from the other. This division, so far as observed, is similar to the first and results in the organization of four nuclei (fig. 13).



FIGS. 1–13.*—Fig. 1, basidium primordium; fig. 2, young basidium; fig. 3, fusion of nuclei in young basidium; fig. 4, basidium with fusion nucleus; fig. 5, cross-section of basidium and fusion nucleus; fig. 6, nucleus with massed chromatic material; fig. 7, material spread out along nuclear membrane; fig. 8, condition after disappearance of nuclear membrane; fig. 9, spindle of first division; fig. 10, same showing dark bodies at poles and two chromatic masses, each with two parts; fig. 11, two-nucleate basidium; fig. 12, second division; fig. 13, four-nucleate basidium.

* All figures drawn at the level of the table with an Abbé camera lucida, under a 1.25 mm. achromatic Spencer objective on a Leitz microscope with a 20× ocular. Magnification about 3850; reduced to two-thirds.

The four nuclei move to the central or basal part of the basidium, where they remain for a short time. At this stage small narrow protrusions, the sterigmata, are evident at the apical part of the basidium. These outgrowths elongate and at the distal end enlarge and



FIGS. 14–19.—Fig. 14, basidium with sterigmata, the four nuclei pushed to apical portion by vacuole; fig. 15, cross-section of basidium with four nuclei connected to sterigmata by cytoplasmic strands; fig. 16, nuclei passing into distal end of sterigmata; fig. 17, mature basidium showing three of the four spores; fig. 18, division of nucleus in spore; fig. 19, binucleate spore.

become spherical. As soon as the sterigmata begin to protrude a large vacuole is formed below the nuclei; the increase in size of the vacuole seems to cause the nuclei to be pushed to the apical region of the basidium (fig. 14). When the nuclei lie close to the apical wall they become fusiform, their membranes become indistinguishable, and often it is difficult to distinguish the nuclei from one another. A

nucleolus is conspicuous at this time in each nucleus; it is surrounded by a homogeneous group of dark granules. A protoplasmic strand connects each nucleus with the portion of the wall at which a sterigma has been formed (fig. 15). Each nucleus moves toward the corresponding sterigma, becomes greatly elongated, and passes through the sterigma into the enlarged distal end of the latter (fig. 16). In moving through the sterigma, the nucleus is stretched to such a degree that it often becomes threadlike at the lower portion; but as soon as it reaches the distal end it begins to thicken and become spherical. The threadlike portion passing through the sterigma becomes narrower and shorter as the part in the distal end becomes broader in diameter. A wall is formed, cutting off the distal part as a spore. A typical nucleus is soon evident in this spore (fig. 17).

Four spores are typically formed on a basidium and only one nucleus passes to each spore. Later the nucleus in the spore divides so that the mature spore is binucleate (figs. 18, 19).

Nuclear behavior in the formation of the spores of *Scleroderma lycoperdoides* is similar to that described by BAMBEKE (1) for *Hydnangium carneum*, and by FRIES (3) for *Nidularia*, although in *Scleroderma* granules which determined the position of the sterigmata were not seen. The present observations do not agree with those of MAIRE (5) for *Scleroderma vulgare* in regard to the centrosomes. MAIRE found that the second division began immediately upon disappearance of the first spindle, while in the present study binucleate basidia have been found with well organized resting nuclei. These observations suggest that the chromosome number is two, as reported by MAIRE for *S. vulgare*.

Summary

1. Fusion of the two nuclei in the basidium primordium of *Scleroderma lycoperdoides* is followed by enlargement of the basidium and of the fusion nucleus. Two nuclear divisions occur. The four nuclei move to the base of the basidium but later are pushed against the apical wall by a large vacuole.

2. The fusion nucleus passes through a stage suggestive of synizesis. The chromatic material becomes stretched out along a spindle, divides, and reaches the poles. The spindle axis in the apical region is transverse to the longitudinal axis of the basidium. Darkly stained

granules are present at the poles of the spindle, suggesting centrosomes. The second division is similar to the first, and the spindles are close to each other in the apical portion of the basidium. There is indication of the presence of two chromosomes in the second division.

3. The nuclei become elongated, and move toward the sterigma and into its enlarged end. There the nucleus resumes its characteristic form and cell division results in the formation of the spore. Each basidium produces four uninucleate spores. Later the spore nucleus divides.

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ANATOMY OF THE TRANSITION REGION IN GOSSYPIUM

CONTRIBUTIONS FROM THE HULL BOTANICAL LABORATORY 449

ALDA MAY SPIETH

(WITH TEN FIGURES)

Introduction

The genus *Gossypium* has been the subject of much investigation from the physiological and anatomical viewpoints. Although much work has been done on the development of the flower and lint fiber, many anatomical details of the vegetative structures require further investigation. This paper describes the root-stem transition region.

Seeds of *Gossypium hirsutum* L. were used in this study, of the upland variety Dixie Triumph. The varieties Acala, Cleveland 54, and Delfos were studied for comparison. The seeds, which were treated with sulphuric acid to limit the development of cotton wilt, germinated rapidly under greenhouse conditions. By the end of the fourth day the hypocotyl may attain a length of 10–12 cm., with a primary root of equal length or longer. In seedlings five days old the cotyledons are fully expanded and the primary tissues are mature in the root and lower part of the hypocotyl. Less mature and older seedlings were used to determine the progressive development and maturation of the tissues.

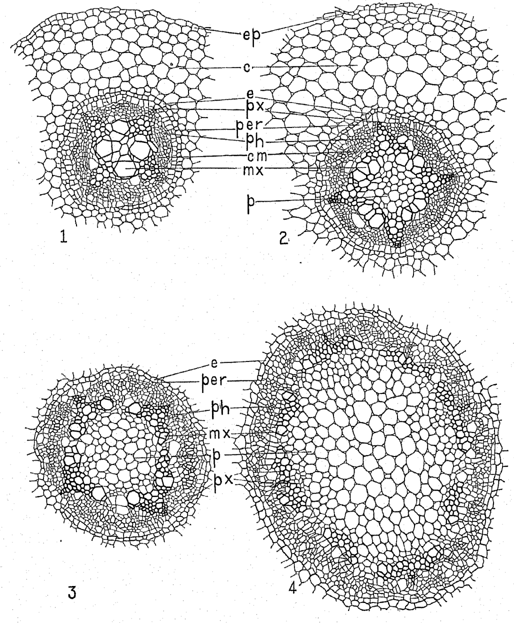
The transition region involves the greater part of the hypocotyl. The xylem is completely endarch approximately 1–2 cm. below the divergence of the cotyledons.

Investigation

PRIMARY ROOT

The primary root of the cotton plant is generally a tetrarch, exarch protostele (fig. 1). The protoxylem is composed of elongated spiral and annular elements, which usually are stretched to such an extent that the thin walls of the annular elements collapse and the spiral bands appear straightened out. The metaxylem adjacent to

the protoxylem consists of scalariform and scalariform-reticulate tracheids; that in the center of the cylinder consists of four or eight large tracheae, which may or may not be separated by cells with



FIGS. 1–4.—Fig. 1, transverse section of primary root: *cm*, cambium; *c*, cortex; *e*, endodermis; *ep*, epidermis; *mx*, metaxylem; *per*, pericycle; *ph*, phloem; *px*, protoxylem. Fig. 2, lower transition region showing pith. Fig. 3, metaxylem separated by parenchyma. Fig. 4, four transition bundles with alternating tangential metaxylem.

scalariform thickenings. The latter elements do not mature until secondary thickening begins.

Alternating with the protoxylem points are four groups of phloem.

These groups are separated from the central metaxylem by parenchymatous cells. The protophloem is parenchymatous and is soon crushed. The metaphloem consists of sieve tubes, companion cells, and parenchyma. When the primary root matures, the walls of some of the phloem cells external to the cambial initials thicken. Thus thickened phloem cells are formed adjacent to the cambial initials. Sieve tubes and companion cells occur at each end of this row of thickened elements.

The pericycle consists of a single layer of cells, external to the phloem region, and of two or three layers of cells over the protoxylem elements. The pericyclic cells are several times the length of their radial diameters.

The endodermis is composed of one layer of cells. The cells abutting on the phloem groups are filled with a mucilaginous substance, but over the protoxylem points this material is usually lacking. The endodermal cells are approximately the same length as the pericyclic cells.

The cortex includes the endodermis and 8–12 concentric layers of parenchymatous cells. These are usually isodiametric, although the two or three rows adjacent to the epidermis may be vertically elongated. The epidermis is a single layer of small cells.

Lateral roots originate early in the development of the primary root. These may be initiated in the pericycle abutting on the protoxylem long before the primary xylem matures.

Root-stem transition

The first indication of a change from the exarch condition of the root to the endarch condition of the stem occurs approximately 1 cm. below the soil level. The hypocotyls that were studied were five days old and were 11–13 cm. in length from the soil level to the point of divergence of the cotyledons. At this age the primary tissues of the lower portion of the hypocotyl are completely differentiated, the cambium layer is active, and secondary thickening is initiated. In the upper portion of the hypocotyl, the primary tissues are beginning to mature. The more meristematic nature of the upper part of the hypocotyl is indicated by the continued elongation of this region and the lack of cambial initials.

At the cotyledonary node there is little differentiation of tissue, indicating that the older part of the axis is the lower portion of the hypocotyl and that the upper portion is much younger.

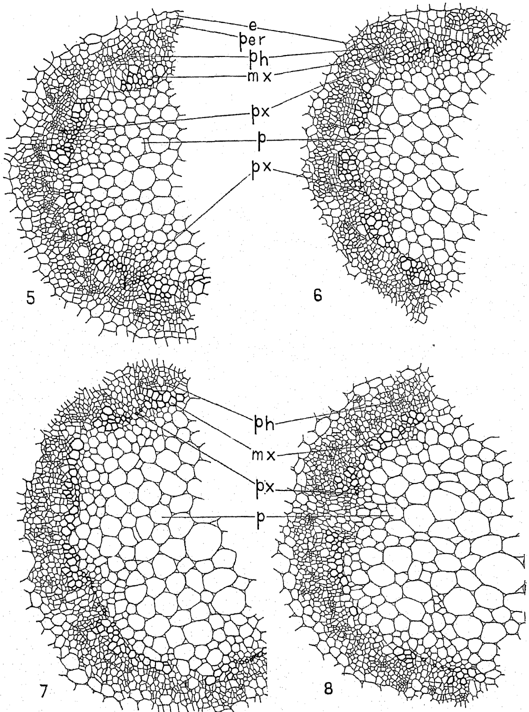
The first change in the root-stem transition takes place where the metaxylem differentiates in a ring, surrounding a pith of parenchymatous cells (fig. 2). The parenchymatous cells of the pith are circular in cross section, with intercellular spaces, and are elongated vertically. The phloem groups are larger, and one or two rows of the cells inside of the pericycle have thickened walls. Sieve tubes and companion cells differentiate at each end of the group, adjacent to the protoxylem points.

Within a short vertical distance the entire stele is enlarged. Four triangular shaped masses of protoxylem and metaxylem cells are laid down (fig. 3). Each triangle consists of the protoxylem at the apex and two tangential arms of metaxylem, which lie opposite the phloem groups and adjacent to the cambial initials.

At a slightly higher level, parenchyma is differentiated between the cells of the metaxylem arms. Four distinct triangular shaped groups of protoxylem-metaxylem elements are formed, with large tracheae at the two inner points of each triangle and with the protoxylem at the apex. Metaxylem continues to differentiate tangentially, so that there are bands of metaxylem alternating with the four transition bundles (fig. 4).

Continuing up the axis, the entire stele becomes larger. The endodermis and the pericycle persist as unbroken bands of cells. The protoxylem points differentiate in the same position, but the triangular shaped masses of metaxylem adjoining each protoxylem point differentiate laterally, so that each row lies at right angles to the protoxylem elements (fig. 5). These four bundles are typical transition bundles. The four large phloem groups are unchanged, except that three or four layers of the cells internal to the pericycle have thickened walls, although the protoplasmic contents are still dense. Small groups of sieve tubes and companion cells may be found at intervals among the thick walled elements.

The next change occurs approximately 2 cm. above the soil level. At this level each of the four original transition bundles consists of two rows of spiral elements separated by parenchyma and two lateral



FIGS. 5–8.—Fig. 5, transverse section of lower hypocotyl showing two transition bundles. Only one bundle shows parenchyma between the protoxylem points. Fig. 6, two transition bundles. In one bundle the protoxylem points are at right angles to metaxylem and are separated by parenchyma; in the second bundle the protoxylem points are in line with the metaxylem elements. Fig. 7, same transition bundles at slightly higher level. Fig. 8, upper transition, showing wide ray between one pair of bundles in contrast to narrow ray of second pair of bundles.

or tangential arms of metaxylem. This condition is first reached in the two opposite bundles. Each of these bundles now consists of two units with the protoxylem in essentially the same position and the metaxylem arranged tangentially. Alternating with these bundles are the other two transition bundles, in which there is as yet no parenchyma between the protoxylem points (fig. 5).

Secondary thickening takes place at this level and the cambium lays down secondary xylem adjacent to the tangential rows of metaxylem. Secondary phloem is differentiated external to the cambial initials. Outside the protoxylem points parenchymatous cells are differentiated by the cambium, and in this way the size of the entire stele is increased.

The endodermis and pericycle retain their identity as continuous bands of cells, but the cortex is composed of 15 to 17 concentric layers of cells. These cells decrease in radial diameter toward the epidermis. At the same level, lysigenous canals may occur in the outer layers of the cortical tissue.

Approximately 4 cm. above the level where transition first begins, the separation of each bundle into two units has been completed. In each unit the protoxylem is still at right angles to the metaxylem. Thick walled pericyclic cells abut on the endodermis, except over the protoxylem points where the pericycle remains parenchymatous (fig. 6). The endodermis appears as a broken band, retaining its identity only over the transition bundles. The cambium remains active and lays down xylem on the inside and phloem on the outside, while over the protoxylem points the secondary tissue remains parenchymatous.

Gradually the protoxylem points of each of the halves of the transition bundles mature opposite each other in line with the row of metaxylem cells (fig. 6). This condition is first noted in two bundles on opposite sides of the axis. In these bundles the protoxylem points are separated by four or five parenchymatous cells. In the bundles alternating with these, the points are more or less at right angles to the metaxylem.

Continuing up the axis, cambial activity decreases and the phloem groups are broken up into smaller units by the maturation of cells as parenchyma (fig. 7). The rays of parenchymatous cells between

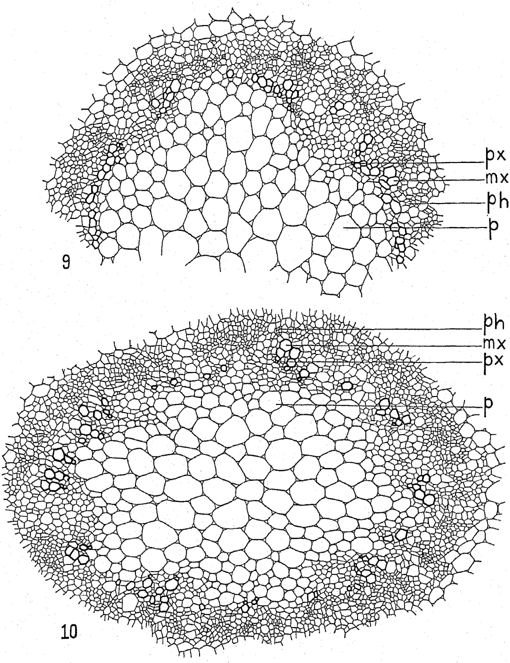
the protoxylem points in the first two bundles to mature increase in size (fig. 8). The pith as a whole remains the same size, but the cells internal to the protoxylem are much smaller in their radial diameters. The cambium at this level is just being differentiated.

The next change is a progressive maturation of the protoxylem inward and a differentiation of the metaxylem outward. Thus by a gradual centripetal differentiation of the protoxylem and a centrifugal differentiation of the metaxylem the endarch condition is reached (fig. 9). This condition occurs first in the opposite bundles. Fewer metaxylem elements mature in each bundle, indicating the more meristematic nature of this region. The parenchymatous rays separating the halves of two bundles become progressively wider, and metaxylem cells appear in the rays. The metaxylem appears progressively, with first one, then two, and finally three cells, each being separated from the other by parenchyma (fig. 9). The rays between the units of the alternate transition bundles remain two or three cells wide. There are no thick walled cells in the phloem at this level, but the sieve tubes and companion cells lie external to the four pairs of transition bundles.

Eventually the endarch condition is reached in all bundles. The rays between the units of the two bundles opposite each other are wide, with two or three endarch bundles in each ray. Alternating with these are the transition bundles in which the halves of each are separated by narrow rays containing no metaxylem elements.

Approximately 8 cm. (in the plants studied) from the soil level, the rays between the units of the two transition bundles that matured first are widened to such an extent that one unit of each bundle is on the opposite side of the axis. Thus four units of the original four transition bundles are on each side of the axis. Each of these groups consists of one original transition bundle, in which the two units are separated by narrow rays, and one unit of each bundle in which the halves are separated by wide rays.

Alternating with the two groups of four bundles are the two or three endarch bundles that differentiated in the wide rays (fig. 10). Each of these bundles consists of a single xylem element and phloem cells. The meristematic character of this level is apparent in the



FIGS. 9, 10.—Fig. 9, two pairs of transition bundles, showing metaxylem element in the wide ray between one of the pairs of bundles. Fig. 10, transverse section of hypocotyl slightly below cotyledonary plate, showing eight endarch bundles. Two pairs of bundles are separated by wide rays, with two or three metaxylem elements in each ray. The other two pairs of bundles are separated by narrow rays.

relatively few metaxylem elements in each bundle and in the undifferentiated character of the phloem region.

At the level of the cotyledonary plate, the four bundles on one side of the axis diverge into one cotyledon. The other four bundles become the vascular traces of the second cotyledon. The remainder of the axis consists of meristematic cells. An examination of the cotyledonary plate of older plants seems to indicate that the endarch bundles, differentiated in the wide rays, become the leaf traces of the axis above the divergence of the cotyledons. The connections of these bundles remain to be determined.

Summary

1. The transition from root to stem in *Gossypium hirsutum* L. is completed in the upper portion of the hypocotyl.

2. The first indication of transition from the tetrarch, exarch, protostelic primary root occurs where the metaxylem differentiates in a ring, surrounding a large pith of parenchymatous cells.

3. At a slightly higher level, parenchyma is differentiated between the metaxylem elements and four triangular shaped groups of protoxylem-metaxylem elements are formed. These are typical transition bundles.

4. Continuing up the axis, the protoxylem elements of each bundle differentiate as two rows of spiral cells, which are formed at right angles to the metaxylem. Eventually these rows are separated by parenchyma. Each transition bundle now consists of two units. At this level a cambium has differentiated secondary xylem and phloem.

5. At a higher level the protoxylem points of two bundles on opposite sides of the axis mature in line with the row of metaxylem elements. Alternating with these bundles are two transition bundles in each of which the protoxylem points are separated by parenchyma, but are at right angles to the metaxylem. Cambial activity has decreased.

6. Farther up the axis there is centripetal differentiation of the protoxylem and centrifugal differentiation of the metaxylem. Ultimately the endarch condition is reached. The rays between the halves of the two bundles that matured first are wide and from one to three metaxylem elements appear in these rays. In the alternate

bundles the rays are narrow with no metaxylem elements. The more meristematic condition of this level is indicated by the relatively few metaxylem elements in each bundle and in the undifferentiated character of the phloem.

7. At the level of the cotyledonary plate, one-half unit of each original transition bundle that matured first, and the two units of the one bundle, in which the rays are narrow, are on one side of the axis. These diverge into one cotyledon. The other bundles become the vascular traces of the second cotyledon.

Grateful acknowledgment is made to Dr. H. E. Hayward for his interest in the progress of this study.

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CURRENT LITERATURE

Freshwater algae

The design and production of an advanced textbook has for no group been a more difficult problem than for the algae, owing to the very great number of plant types concerned and to a supposedly limited market. It was solved in England by G. S. West in Algae, Vol. I for a general text on morphology and life history, and in British Freshwater Algae for classification with a more limited amount of general information. Smith has now prepared an excellent text[1] based on the United States flora (to our knowledge of which he has notably contributed) with an extraordinarily full treatment of the morphology and life history of each genus. As the genera are nearly all illustrated, usually by original figures and often in both vegetative and reproductive stages, and as keys to the families and genera are provided, the book is an effective introduction to classification, thus combining the valuable features of its British predecessors. In rare instances the illustrations and text do not seem in full agreement (for example, the figure of *Hormidium* and its generic key character, p. 379). Lacking complete descriptions, and, in all but the smaller genera, citation of more than a few representative species, it is obviously not intended as a taxonomic manual. It would have been of advantage had the author given for each major genus one or more references to fairly comprehensive taxonomic sources, so that the student could quickly reach a just idea of the criteria used in species delimitation. In fact, it will be difficult for the beginner to select from the extensive bibliography that indispensable group of papers without constant access to which he could not expect to accomplish reliable classification of his material. Since the taxonomist must consider that this book is strictly introductory, the reviewer would deprecate the introduction of a considerable number of new names, combinations, and species which to him seem out of place in a general textbook.

Since the approach to the subject is through the American flora, this book is not a general textbook of algal morphology. The morphological material is organized on a systematic basis, and the systematic scheme adopted is conservative. In the case of the Myxophyceae the arrangement is in marked contrast to the most recent general treatment, an elaborate one devised by Geitler to cover the much more extensive flora with which he dealt. In many groups the student will find novel rearrangements of the material, most of which have good foundation. In the Rhodophyceae it does not seem to the reviewer that placing *Compsopogon* in the Erythrotrichiaceae can be sustained, for against the similarity in method of spore production are great differences in cell structure (a very

[1] Smith, G. M., The freshwater algae of the United States. pp. xi+716. figs. 449. McGraw-Hill. New York. 1933.

conservative character) and in thallus organization, and the fact that the genus in question is strictly freshwater while its associates in the family proposed would all be marine. Development of the systematics of the Heterokontae, Chrysophyceae, and Chlorophyceae in general enabled the author to incorporate advantageously the rapidly accumulating information on life histories. Separation of the Ulotrichales from the Ulvales by the Oedogoniales would seem to obscure the obvious transition between the first mentioned orders, while the Oedogoniales show no immediate connection with either of the others. Association of the Cryptophyceae with the Dinophyceae in a division Pyrrophyta is accepted, but the Cryptophyceae are omitted as not having an algal type of organization; it would have been advantageous to have developed briefly the characters of the group and its relations with the Dinophyceae, in order that the characteristics of the whole division might have been made clear. Even if their more advanced representatives do not retain algal characters, their common origins with algal types justify at least introductory treatment. The Charales are excluded from the book without explanation, hardly an advisable move in view of the wide belief among phycologists in their algal nature. The great ecological importance of the group makes this lack a serious one, and their physical association with algae encourages their study jointly with other algal types.

These features of organization and content, to which the reviewer has drawn attention, do not seriously detract from the very great value of this text, or its ready usefulness in advanced instruction. It will certainly do much to eliminate ill-informed and superficial study and teaching of algae from American laboratories, and the country is fortunate that its first algal textbook is such a good one.—WM. RANDOLPH TAYLOR.

Wild flowers of the Alleghanies

In the eastern United States no region surpasses the Alleghanies in botanical interest. There has recently appeared a charmingly written and scientifically accurate work on the wild flowers of this region. In this book by HARNED[2] appear many interesting features, some of which are unique in a book of this sort. Perhaps the most unusual feature is the illustrated key to the families, prepared by MATHEWS, as are the numerous other excellent illustrations of characteristic species and organs. The eight beautiful colored plates were prepared by MATHEWS and EATON. The author designed the volume for the technical botanist and the non-technical flower lover, and has succeeded remarkably well in meeting the needs of these two divergent classes. The specialist will notice the omission of a number of genera and families that would not particularly attract the botanical novice or the non-technical flower lover; among such omitted groups are the grasses, sedges, rushes, pondweeds, willows, and the amentiferous trees.

[2] HARNED, J. E., Wild flowers of the Alleghanies. 8vo. pp. xxxii+670. pls. VIII. figs. 400. Published by the author. Oakland, Md. 1931.

In addition to the excellent keys and description of species, the book contains many interesting and important field data, including notes on habitat, color, and agents of pollination. Generally through the volume plant uses are mentioned, especial attention being given to medicinal uses. In many cases the legendary history of interesting species is portrayed. Obviously the author is a field botanist and a nature lover of rare enthusiasm, whose spirit is sure to be imparted to those who read and use the volume. As would be expected, the author is an enthusiastic conservationist, and makes a strong plea for the preservation of our wild flowers. He is to be congratulated on the publication of a work that in content and typography may well be taken as a model of its kind.—H. C. COWLES.

Handbook of plant analysis

For several years the Handbuch der Pflanzenanalyse by G. KLEIN has been going through the press of Julius Springer in Vienna. The earlier volumes have been noted previously in this journal.[3] The final volume[4] has now been published in two large sections paged consecutively.

The first half contains 830 pages, the second 1030. In the first half the methods presented include those for amino acids, amides, amines, betaine, cholin, muscarin, proteins, purines and pyrimidines, nucleins, alkaloids, and cerebrosides. Brief consideration is also given to some little known compounds that include the so-called bitters, etc. The second half takes up enzymes, fermentation methods, antigens and antibodies in plants, analysis for plant hormones, and vitamines. Accompanying the section on enzymes there is a table giving the systematic distribution and occurrence of enzymes in plants. The work closes with a presentation of some special techniques. Biological methods of analysis for certain compounds are presented, such as the digitalis group of alkaloids, aconitin, nicotin, coniin, atropin and other solanaceous alkaloids, physostigmin, morphine, strychnin, caffein, picrotoxin, veratrin, and colchicin. Many of these tests involve the use of frog muscle reactions. Another section is devoted to the analysis of natural waters. Methods for soil analysis, analysis of fermentation media, nitrogen fractionation of plant materials, and the fractionation and purification of pigments by chromatographic adsorption are included among the special methods. An extensive table (pp. 1442–1707) gives certain physical constants for plant constituents. The general formula, melting point, boiling point, solubility, and optical activity are included, as far as they have been determined, for a vast assemblage of known compounds.

The work as a whole is an impressive monument to the industry and patience of the authors, editor, and publishers, who have spent years of effort on it. The user must not expect to find much critical help in the choice of methods to be used with special types of material; nor will he find aid in critical estimation of

[3] BOT. GAZ. **92**:332–333. 1931; **94**:427–428. 1932; **94**:832–833. 1933.

[4] KLEIN, G., Handbuch der Pflanzenanalyse. 8vo. pp. xii+1868. Springer. Vienna. 1933.

the value of the methods. Those who use the work will find it necessary to determine the degree of accuracy, and to consider necessary modifications of methods to take care of precautions to insure accuracy and dependability of the results. It is impossible to have tested the methods critically before compiling them.

The cost of the work, especially at present rates of exchange, makes it prohibitive for the individual. The quoted prices for the entire set in brochure binding is RM 514 (somewhat less than $200) to the American investigator or library. This is significant evidence of the increasing costs of maintaining adequate facilities for the continuation of investigations under our present methods of organization.—C. A. SHULL.

Alpine succession

Plant communities of James Peak, Colorado, have been studied by Cox,[5] who, besides shorter studies in other years, spent two entire growing seasons (1928 and 1929) in the field, employing modern ecological methods. Cox's station for the observation of temperature was at the lower boundary of the alpine zone, 11,300 feet altitude, while his vegetation studies were carried to the top of the peak, 13,345 feet. The mean annual temperature as well as the temperature for the summer months suggests the Arctic Circle. Frosts occur every month, and most frequently in the higher parts of the zone studied. Floristic censuses were made with 0.5-meter quadrats in meadow and fellfield, and 10-meter quadrats in scrub and timber. The number of quadrats studied in each plant community is so large that the averages of frequency and ground covered must show a high degree of accuracy. An alpine mesophytic meadow climax, with the sedge *Elyna bellardi* dominant, develops in the alpine zone. The successional stages by which this is reached, from bare rocks on the one hand and from pools and wet areas on the other, is carefully traced. Recognized associations and edaphic climaxes include those of fellfield, sedge moor, and meadow with their various minor communities. Species of vascular plants to the number of 197 are recorded from the alpine zone. The chief families with the number of species in each are: Poaceae 21, Cyperaceae 23, Caryophyllaceae 11, Ranunculaceae 8, Brassicaceae 9, Saxifragaceae 9, Rosaceae 10, Scrophulariaceae 10, Carduaceae 28. Since very little detailed ecological work has hitherto been done in alpine regions of America, this study is destined to be rated as a classic to which reference will needs be made in future investigations.—FRANCIS RAMALEY.

Galls

Galls have fascinated both botanists and zoologists since the days of MALPIGHI. The subject matter of cecidology is of great interest to the botanist, not only in its anatomical but also in its physiological, ecological, and pathological aspects. For ecologically minded zoologists, it is of perennial interest because of

[5] Cox, C. F., Alpine succession on James Peak, Colorado. Ecological Monographs 3:299–372. 1933.

the symbiotic association of plant and animal. ROSS, who has devoted more than 30 years of study to galls, has published the results of his study in an attractive volume which is a signal contribution to the field of cecidology.[6] The volume consists of two parts: a general part of 25 pages devoted to the basic concepts, propositions, and definitions of cecidology; and a special part, devoted to concrete material illustrating the general principles. Twenty-four chapters are devoted to examples of galls selected on the basis of form and origin. Nine chapters are devoted to Cynipid galls. In addition, one chapter is assigned to mycelium-infected insect galls, and an appendix treats the technique of collection and storage of galls as well as culture and identification of gall incitants and the season during which material is collected most advantageously. The volume includes an excellent bibliography and index.—G. K. K. LINK.

Cytology

SHARP'S textbook on cytology has found such favorable reception in Germany that the second edition has made its appearance as a German translation.[7] The volume is more than a translation; it is a completely reworked, revised edition. The additions and changes made by the translator are marginally indicated. Most changes and departures from the original occur in the discussion of protoplasmic inheritance, influence of genes, mitogenetic rays, the nature of spindle fibers, etc. Three hundred titles of new pertinent literature are added to the excellent bibliography of the second American edition, and the data and concepts of these contributions are integrated into the whole. In the main the translator has carried out a difficult task satisfactorily. The volume is not only a valuable addition to the working library of German botanists, but the additions will render it of service to American and English botanists until a new edition is produced by the author.—J. M. BEAL.

Plant geography

A noteworthy contribution to the plant geographic conditions of Mediterranean countries is a recent study of the vegetation of Albania.[8] The author does not merely describe the vegetation of a small country, but also discusses its relation to the Mediterranean and Balkan floras. A large floral map of Albania is included.—A. C. NOÉ.

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[6] ROSS, H., Praktikum der Gallen Kunde. pp. viii+312. figs. 181. Julius Springer. Berlin. 1932.

[7] SHARP, L. W., Einführung in die Zytologie. (Trans. by R. JARETZKY.) pp. iv+733. figs. 212. Bornträger. Berlin. 1931.

[8] MARKGRAF, F., Pflanzengeographie von Albanien. Ihre Bedeutung für Vegetation und Flora der Mittelmeerländer. Bibliotheca Botanica No. 105. E. Schweizerbart'sche Verlagsbuchhandlung. 4to. pp. 132. Illustrated. Stuttgart. 1932. RM 56.

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THE
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VEGETATION OF THE GREAT SALT LAKE REGION

CONTRIBUTIONS FROM THE HULL BOTANICAL LABORATORY 450

SEVILLE FLOWERS

(WITH ONE FIGURE)

Origin of Great Salt Lake

Great Salt Lake is located in northwestern Utah and is the remnant of Lake Bonneville, a great body of fresh water extant in Pleistocene times. At its highest level the ancient lake stood about 1000 feet above the present level of Great Salt Lake. A slight rise in level caused it to overflow its rim at a point since known as Red Rock Pass, Idaho. The outlet was eroded at a rapid rate with the subsequent lowering of the lake level. Erosion continued for 375 feet, when a large mass of hard rock was encountered. At this time the lake is believed to have had a longer period of constant level than at any other period in its history. Further decline of the lake was due to evaporation. At a point 200 feet above the present level of Great Salt Lake, alkaline salts began to concentrate and were deposited in the sediments. Evidences of the extinct lake are manifested mainly in the terraces and alluvial fans high along the bases of the mountains. Two large terraces are very prominent, the Bonneville terrace, which marks the highest level, and the Provo terrace, which was formed when the rapid erosion of the outlet became arrested.

Physiography and geology

The topography of the region presents a series of parallel mountain ranges and valleys extending north and south, and cut diag-

onally by a series of passes so as to impart a somewhat latticed design to the region as a whole. Great Salt Lake occupies an area of coalescence of valleys and passes. To the east of the lake the Wasatch Mountains rise and constitute one of the principal watersheds of the basin. The Oquirrh and Stansbury mountains are short ranges lying directly south of the lake.

The greatest extent of the lake shore is bordered by lowlands. The valley floors are composed of lacustrine sediments dominated by clays and loams. The depth of the valley fill has not been determined definitely, but wells have been sunk to a depth exceeding 2000 feet and the deposits have been found to be remarkably similar, both vertically and horizontally. Extending from the lake the following soil series is recognized: Great Salt Lake sand, Jordan clay, Jordan sand, Jordan sandy loam, Jordan loam, Bingham loam, and Bingham gravelly loam. The Jordan series is notably alkali-bearing.

The mountains rise abruptly from the floor of the valley in many places, but long alluvial slopes, called bajadas, extend for 1 to 5 miles, gradually rising to an elevation of nearly 1550 feet above the bottom of the valley. Old lake terraces high along the bases of the mountains are a conspicuous topographical feature.

Practically all of the permanent streams reaching the lake arise to the east. Three rivers, the Bear, Weber, and Jordan, carry the greatest amount of drainage water. Numerous smaller streams enter directly. The southern, western, and northern sections contribute almost no surface water other than flood run-off. Springs are common along the bases of the mountains and along the lake shore, while permanent wells have been sunk at various points.

Climate

Precipitation and temperature data were taken from representative stations surrounding the lake. Additional data were obtained from other stations. Stations located near the base of high mountains show the highest average rainfall while the valley and desert stations show the lowest amounts. Corinne, Ogden, Farmington, and Saltair are located on the eastern side of the lake, Midlake is on the Southern Pacific Railroad trestle in the middle of the lake, Kelton is located at the northwestern extremity 7 miles from the shore, and

Lemay is 20 miles west of the lake on the Great American Desert. The eastern section averages between 16 and 17 inches of rain per year, Farmington showing the highest annual mean of 20.67 inches and Corinne showing the lowest mean with 12.56 inches. The annual precipitation of the western section averages about 5.0 inches. Midlake shows 5.39 inches. Assuming that the data from these stations approach the averages of each section, there is about 10 inches difference between the western and eastern sides of the lake. The mean annual rainfall data are included in table I. Fragmentary data show

TABLE I

MEAN ANNUAL PRECIPITATION, TEMPERATURE DATA, AND LENGTH OF GROWING SEASON FROM GREAT SALT LAKE REGION

Station	Precipitation (inches)	Temperature (°F.)	Length of growing season (days)
Farmington	20.67	49.8	....
Ogden	15.20	52.2	154
Corinne	12.56	50.4	....
Saltair	14.51	50.7	193
Kelton	6.44	48.4	108
Lemay	4.32	51.1	174
Midlake	5.39	52.2	225

that the annual average for the mountains above 9000 feet elevation amounts to something between 28 and 40 inches. This is significant, as the contribution to the lake by way of streams depends upon the rainfall in the higher ranges.

The mean annual temperatures of the region show little variation and range from 48.4° to 52.2° F. The same stations furnished this datum as shown in table I.

There is a wide variation in the dates of the last killing frost in the spring and the first killing frost in the autumn. The averages of the dates recorded at each station furnish the approximate length of the growing season as shown in table I.

The only station within range of the lake where records of the relative humidity have been kept is located in Salt Lake City. The daily variation is very wide in the summer, ranging from 46% at 6:00 A.M. to 26% at 6:00 P.M. Table II shows the average monthly and annual percentages. Evaporation and wind movement data (table III)

were obtained from Midlake and Lehi, the latter station being located 25 miles south of Great Salt Lake.

According to the records taken in Salt Lake City, the sun shines 62.4% of the time possible for it to do so. The heaviest average amount occurs in July, with 80%. The average wind velocity in Salt Lake City is 7.8 miles per hour; the prevailing direction is southwest. The averages recorded here are taken from the records of the

TABLE II

AVERAGE MONTHLY AND ANNUAL HUMIDITY PERCENTAGES COVERING 31 YEARS IN SALT LAKE CITY

	Jan.	Feb.	Mar.	Apr.	May	June	July	Aug.	Sept.	Oct.	Nov.	Dec.	Annual
6 A.M.	75	73	65	69	57	50	46	46	49	58	65	73	59.7
6 P.M.	70	64	50	39	36	28	26	26	32	45	58	69	45.2

TABLE III

AVERAGE MONTHLY AND SEASONAL EVAPORATION IN INCHES AND WIND MOVEMENT IN MILES

	Apr.	May	June	July	Aug.	Sept.	Oct.	Nov.	Season
Lehi									
Evaporation	5.533	8.583	9.957	10.412	9.455	6.776	3.887	1.502	56.752
Wind	3,972	4,119	3,664	3,039	3,161	2,633	2,671	2,433	29,458
Midlake									
Evaporation	6.815	11.888	8.310	14,369	13.612	6.908	5.753	3.888	68.677
Wind	7,794	9,204	8,030	7,731	5,945	7,983	6,306	5,727	69,139

Weather Bureau of the United States Department of Agriculture at Salt Lake City. With the exception of the evaporation data these records extend from 1879 to 1930.

Great Salt Lake

The actual shape and dimensions of Great Salt Lake are matters of no stable mean. The fluctuations in level from season to season and year to year alter the outline of the shore very materially and to a certain extent the dimensions. In general the long axis has a northwest-southeast trend. Its approximate size, according to the U.S. Geological Survey in 1906, is 75 miles long and 35 miles wide. The mean depth in 1929 was about 15 feet. The shape as represented on

the official maps of the Geological Survey shows it at its highest level, which was recorded in 1868. At that time the mean depth was 19 feet and all of the beaches exposed at the present time were submerged by at least 0.5 foot of water. This old shore line is well defined by a terrace ranging from 1 foot or less to 10 feet high. Throughout the ensuing discussions this shore line will be referred to as "the old shore line" or "the shore of 1868."

The deepest part of the lake is found in a trough which runs northwest-southeast between Antelope and Stansbury islands. The records of 1869 give the greatest depth as 49 feet; and with the great recession of 1903 measurements in a similar place showed only 36 feet, a difference of 16 feet, which corresponds to the difference in surface measurements.

The periodic oscillations have been measured over a period of nearly 75 years and on the average show a difference of 2 feet from the wet to the dry period. The level also fluctuates non-periodically chronologically. The level was first measured in 1850. In 1862 a permanent gauge was set up from which monthly readings were taken. Since records have been kept the lake has had one noticeable advance, followed by a recession, and in turn by another less pronounced advance; at present it is sinking again. The following are the important fluctuations:

1850......	3 feet above the zero on gauge
1868......	14 feet above the zero on gauge
1874......	8 feet above the zero on gauge
1877......	14 feet above the zero on gauge
1903......	2 feet below the zero on gauge
1923......	8 feet above the zero on gauge
1931......	1 foot above the zero on gauge

The zero on the official gauge at Saltair stands at 4194.8 feet above sea level.

CHEMICAL CONSTITUENTS OF THE BRINE.—The waters of the lake have been analyzed repeatedly and the results are shown in table IV (2). The brines vary from 15 to 27.6% according to the level. In comparing the percentage of salts in the water with the fluctuation in level, it is found that there is approximately 1% increase in concentration for every foot decrease in level. This is relative, however,

since a very rapid recession of the lake often causes the salts to be precipitated in great masses of crystals instead of increasing the concentration very much. There are two sources of the salt, concentration of water from streams and contribution from hot mineral springs. The latter are 1–5% salt.

TABLE IV

ANALYSES OF GREAT SALT LAKE WATER (2)

	A	B	C	D	E	F
Cl	55.99	56.21	56.54	55.23	55.11	53.72
Br	Trace	....	....	Trace	....	....
SO_4	6.57	6.82	5.97	6.73	6.66	5.95
CO_3	....	0.07	....	....	....	....
Li	Trace	....	....	Trace	....	....
Na	33.15	33.45	33.39	34.65	32.97	32.81
K	1.60	(?)	1.08	2.64	3.13	4.99
Ca	0.17	0.20	0.42	0.16	0.17	0.31
Mg	2.52	3.18	2.60	0.57	1.96	2.22
	100.00	100.00	100.00	100.00	100.00	100.00
Percentage salinity	14.904	13.790	19.538*	27.720	22.990	17.680

A. ALLEN, O. D., Rept. U.S. Geol. Expl. 40th Par. **2**:435. 1877. (Water collected 1869.)
B. SMART, CHARLES, cited in Resources and attractions of the Territory of Utah. (Collected 1877.)
C. TALMAGE, J. E., Science **14**:445. 1889. (Collected 1885.)
D. BLUM, W., Reported by TALMAGE in Scot. Geog. Mag. **20**:424. 1904. (Collected 1904.)
E. EBAUGH, W. C., and WILLIAMS, K., Chem. Zeitung. **32**:409. 1908. (Collected 1908.)
F. MACFARLANE, W., Science **32**:568. 1910. (Collected 1910.)

In comparison with other bodies of saline water, Great Salt Lake ranks among the most highly concentrated. The following tabulation shows the percentages of some of the saline lakes of Eurasia (3).

	Percentage salinity
Caspian Sea	1.294
Sea of Aral	1.084
Dead Sea	19.2 to 25.99
Red Lake, Perekop, Crimea	30.01
Gaukhane Lake, Persia	25.88

RECENT CONDITIONS.—The period extending from 1929 to 1931 exemplifies rather typical behavior of the lake. During the former year the level was 4–6 feet above the zero on the gauge and within 2 years receded almost to the zero mark, averaging about 1 foot above. It is obvious that bays and inlets fed by fresh-water tributaries have much less salt than the main body of the lake. Table V

TABLE V

PERCENTAGE TOTAL SALTS AND H-ION CONCENTRATION OF BRINES AT VARIOUS POINTS IN LAKE

Locality	Date	Percentage salt	pH
Main body:			
Near Saltair	June 12, 1929	18.04	
Near Saltair	Aug. 30, 1931	20.50	8.2
North Point	June 12, 1929	18.06	
Near Jordan delta	July 29, 1929	15.71	
West Point	July 24, 1929	17.98	
West Farmington	Sept. 3, 1931	21.70	8.6
Little Mountain	June 29, 1929	18.01	
Bays and inlets:			
East Bay in swamp	June 17, 1929	1.03	
East Bay outer limits of swamp	June 17, 1929	5.86	
East Bay at Jordan delta	Sept. 8, 1931	10.60	8.2
Willard Spur, south side	July 8, 1929	11.68	
Willard Spur, north side	July 8, 1929	8.60	

gives the percentage total salts and H-ion concentration of water taken from representative situations.

The H-ion concentration of the brines is much the same in all parts of the lake. It is perhaps lower than might be expected in such a highly concentrated solution, but the strong buffer action of the weaker acid radicals checks the amount of dissociation. Even fresh waters of the same region have a pH of 8.2, so that there is little or no effect on this value when added to the lake waters.

Flora of Great Salt Lake

GENERAL FEATURES.—The water of Great Salt Lake is clear and transparent. In appearance there is nothing to distinguish it from a body of fresh water. Occasionally masses of algae form local colonies, imparting a brownish tinge to the water, and except in the bays

and inlets no emergent plants grow in it. The high saline constituents render the water toxic and unavailable for the use of most plants. On the lake borders and saline plains trees are absent, succulent herbs and low shrubs predominating. There are a few algae, lichens, and mosses but no liverworts nor pteridophytes. Hackberry and junipers make their closest approach on cliffs and sand dunes, willows occur along the rivers, and poplars have been introduced.

On the basis of salinity the flora of the lake waters may be divided into two general categories, that of the main body of the lake and that of the bays, inlets, and springs.

FLORA OF MAIN BODY OF LAKE.—Algae are the only plants that normally inhabit the main body of the lake where the salt reaches a high concentration. The following were collected:

Myxophyceae:
- Aphanothece utahensis Tilden[1]
- Microcystis packardii Farlow
- Oscillatoria tenuis var. natans (Kuetz.) Rab.
- O. tenuis var. tergestina (Kuetz.) Rab.

Chlorophyceae:
- Chlamydomonas sp.
- Tetraspora lubrica var. lacunosa Chauv.

Aphanothece utahensis is the dominant species at the present time. It floats on or near the surface in flaccid, rugose brown masses 4–6 inches in diameter. In local places it sometimes accumulates, imparting a brownish tinge to the water. It is rather evenly distributed in the deeper parts of the lake but is in greatest abundance along the eastern shore, on the eastern side of Stansbury Island, the northern and western sides of Antelope Island, and on the steep shores of the western side of the promontory. *Microcystis packardii* occurs in rather firm gelatinous masses 0.5–1 inch thick, usually spherical but often irregular in shape. The color is most frequently blue-green but brown and pale yellowish tints are common. The species of *Oscillatoria* are much less common and were encountered only occasionally.

[1] The manuals used in the identification of the plants mentioned in this paper were RYDBERG's Flora of the Rocky Mountains, TIDESTROM's Flora of Utah and Nevada, COULTER and NELSON's Manual of Rocky Mountain Botany, LESQUEREUX and JAMES' Manual of North American Mosses, and BARNES and HEALD's Key to the Genera and Species of Mosses.

All of the collections came from the eastern shore, where it forms thin layers on the muddy bottom in the still and shallow water. *Chlamydomonas* is abundant and fairly evenly distributed in the deeper parts but most common along the shores mentioned in connection with *Aphanothece*. *Tetraspora* floats in the brine along the quiet shores, growing in slimy olive-green masses. It is not common. Several diatoms were collected in the main body and a great many were taken from points where fresh water enters. Identifications were not obtained, but of the former group DAINES (4) says that two of them probably belong to the genera *Navicula* and *Cymbella*. DAINES also isolated in pure cultures five bacteria, four of which were bacilli and one diplococcus. No names are given.

Many other species of algae were taken from the main body of the lake, but the evidence seemed to indicate that they had been washed in from fresh-water situations. Among these are species of *Cladophora*, *Ulothrix*, *Spirogyra*, *Rhizoclonium*, *Enteromorpha plumosa*, and several others that could not be identified. PACKARD (9) reported the first account of the algae in Great Salt Lake in 1879. FARLOW identified the collections, among which he reported *Ulva marginata* Ag. and *Rhizoclonium salinum* Ktz. TILDEN distributed a series of algae from the lake which included *Enteromorpha marginata*, *E. tubulosa*, *Dichothrix utahensis*, and *Chara contraria*. In the present investigation none of these algae was found to be established inhabitants of the main body of the lake, but they grow abundantly in springs and brackish pools along the shore where the salt is greatly reduced in concentration.

FAUNA.—There are three species normally living in the lake water, the brine shrimp, *Artemia gracilis* Verrill, and two salt flies, *Ephydra gracilis* Packard and *E. hians* Say. These animals feed on the algae and are important in connection with the shore deposits.

EFFECTS OF ORGANISMS ON SEDIMENTATION.—The deposition of carbonate is of interest since the origin of this radical was obscure in view of the fact that the ordinary procedure of analysis of the lake water showed no carbonate radical. CAMERON (11) explains the cause of this as follows:

There is sodium carbonate in solution, and normally this would dissociate with the formation of sodium ions, which could be detected with phenolphthalein but the water contains so large amount of salts with sodium ions that the solu-

tion is saturated with respect to this ion, and, carbonates being salts of a weak acid—that is, with a relatively small tendency to dissociate—the dissociation of the sodium carbonate is completely "driven back." Consequently there is no dissociation in the solution.

If the lake brine is diluted with distilled water, more sodium ions are permitted to enter solution, including those of sodium carbonate. At the same time the ions of the carbonate radical are permitted to go into solution and their presence may be detected with phenolphthalein.

The slightly soluble calcium sulphate occurring in the lake furnishes the calcium ions in the formation of calcium carbonate. This takes place by the forced solution of sodium carbonate resulting from the interaction between sodium chloride and calcium sulphate. The calcium and chlorine ions form calcium chloride, which is very soluble. This increases the solubility of calcium by permitting more calcium sulphate to enter solution. The calcium ions increase beyond their limit of saturation and tend to unite with the carbonate radical. The calcium carbonate is deposited on sand grains forming oolite or upon masses of algae, principally *Aphanothece* and *Microcystis*, forming a tufa-like deposit. In the latter case algae accumulate in shallow water and become cemented down to the increasing bed of tufaceous deposit. This is particularly evident along the shores of the islands and the promontory. Another type of deposit composed of a combination of oolite and tufaceous accumulations is evident in many places. Masses of algae together with the empty nymph shells of the salt fly are washed up on the shore and become incorporated with the grains of oolite. Repeated washing by the waves leaves more and more calcium carbonate in this heterogeneous mass. In this condition the algae become partially disintegrated, forming a mushy mass 4–6 inches deep, the color varying from the naturally bluish green and brown to light tints of pink, yellow, and hyaline. Acres of this gelatinous material are common along the eastern shore. As the lake recedes this material is left exposed on the beach and gradually dries out, resulting in the formation of crusty biscuit-like deposits 1–2 inches thick and 1–3 feet in diameter. These masses are built upon one another and become covered with wind-blown oolite. In time they are transformed into a friable platelike rock resembling

disintegrating sandstone. Older deposits show a thin undulated tufaceous rock, white to pale yellow in color and weathering into small plates. Hardpan and other calcareous deposits have been found in Jordan Valley a few inches to 10 feet deep and 5–10 miles from the present shore of the lake, indicating that this process of rock formation has been taking place over an extended period of time. Along the eastern shore, from the Jordan delta to the mouth of the Weber River, silt together with the vegetable matter becomes incorporated with the algal masses and is turned black by the action of sodium carbonate. As the lake recedes it is left exposed, the surface variegated and apparently solid, but really soft and gelatinous, 6–10 inches deep, and jet black. As the hot sun plays upon it, it puffs up and emits gases of strong sulphurous briny odor. The crusty formations are much the same as those just described, except that they are black with a hoary coat of salt.

While the flora of the lake waters is very limited in number of species of algae, it exerts an important influence on the shore deposits and is directly instrumental in the formation of a specific type of substratum upon which other plants encroach. The ecological significance of the algae becomes two-fold, first, as inhabitants of one of the most highly concentrated natural brines, and, second, as the agents of deposition.

Strand

The term "strand" as used in this paper includes any ground left exposed between the margin of the water and the highest level attained by the lake since records have been kept. This level is well defined by the old shore of 1868. The lateral extent may vary from a few feet near the headlands to nearly 2 miles on the flat beaches. The bars between the mainland and Antelope and Stansbury islands are often exposed. Seasonal variations alter the strand greatly.

The strand deposits may be divided into three groups: sand, oolitic limestone, and mud. The usual conditions show a laminated character of alternating layers of gray, black, and greenish black sediments. The coloring is due to the decay of algae and finely divided vegetable matter brought in by the rivers. Mud and silts occur mainly near the inlets and river deltas. Within a given area the fineness and uniformity of sand and mud may be continuous over a

rather extended range. Where hardpan is in process of genesis a coarser element is introduced.

FLORA.—For the most part the strand is barren. Trees are absent and shrubby plants rare. The pioneer vascular plants are restricted to the extreme margins and occasionally are scattered in isolated groups on the salt flats. The principal types invading the highly concentrated alkali soils may be considered briefly.

The samphires, *Salicornia rubra* and *S. utahensis*, are the first pioneer types of the halosere of Great Salt Lake. They tolerate a higher percentage of alkali in the soil than do any other species. *S. rubra* is an annual plant with opposite branches and minute scale leaves. It is a singular feature that the higher the salt content becomes the shorter and more densely crowded these plants grow. At the very margin of invasion stands 2 inches high are so dense that it would seem impossible for another plant to enter among them. In some places it may be seen to cover a strip of outer strand 30 feet wide, and again it may be absent. In mildly saline soil it reaches a maximum height of 12–14 inches. *S. utahensis* is an endemic plant, perennial, and more robust than the red species. It forms dense caespitose colonies and develops little hummocks of wind-blown material.

Second only to the samphires in toleration of alkali and in its habit of invading is the pickleweed, *Allenrolfea occidentalis*. It is a perennial which becomes shrubby at the base. The stems and leaves are much like those of the samphires but are characterized by alternate branching, and the plant as a whole is much larger and with a different habit of growth. It is common in certain parts of the strand region, invading barren flats, building up little hummocks of wind-blown sand and dust. The annual inkweed, *Suaeda erecta*, is another plant prominent in invasion of the strand. It is less tolerant of alkali and generally follows the samphires and pickleweed. A perennial species, *S. moquinii*, is of more robust habit and often follows the former species. The salt grass, *Distichlis spicata*, is perhaps the most versatile plant in the region. It usually follows the preceding species in the succession, but also enters the strand directly, sending out long rootstocks with the individuals arranged in straight lines.

Less common is *Atriplex hastata*. It is also very versatile though

never attaining any degree of dominance. In soils bearing a moderate amount of alkali and which are dry it is rather small, slender, and silvery gray in color. In very moist or wet soils it becomes tall, branched, and with dark green fleshy leaves. The two types were once regarded as separate species. *Scirpus americanus* and *S. paludosus* are swamp plants tolerant of alkali. They enter the strand at fresh-water inlets, bays, and deltas where the strong alkali is somewhat reduced.

While halophytes become smaller in size the more highly concentrated the salt becomes, the opposite extreme is noted in less saline situations where they reach a maximum size. If, however, the alkali content of the soil becomes less than the optimum, the plants become smaller and more spindling. This situation is seldom observed in nature since competition with other plants soon eliminates them. An example of this is noted in river deltas where the rushes eliminate the samphires.

A point of particular interest is the water relationship of these plants. While the data may tell the percentage salt in soil samples when dry, they tell little regarding the actual conditions while the plants are growing in them. This is due mainly to the varying amounts of water. It has been noted that samphires grow in soils bearing as high as 6.5% salt, calculated from the dry weight of the soil. It is important to note, however, that such soils as harbor samphires have a relatively high water content and that the real feature of significance is the concentration of the soil solution. In the spring the water table is high and the sands and clays are saturated, the concentration is reduced, and germination and growth are initiated. Once started, and the plants having become established, subsequent withdrawal of the water is common. The reaction of the plants differs according to the amount of salt present and the type of soil. Plants persist longer in dry sandy soil than they do in dry clay. The rapidity with which the soil is dried is also a condition bringing about variable plant reactions. In both sandy soil and clay, the slower the drying process the more tenaciously the plants persist, even after the soil becomes very dry and cracked. If the soils are dried out rapidly, however, the plants perish even while there is ample water available.

Several samples of soil were taken from the root region of various typical halophytes and analyses made for water content, total salts, and in some instances for basic constituents. The salts were extracted with excess distilled water, the liquid passed through a porcelain filter, and the filtrate used in standard quantities for analyses. The H-ion concentration was determined with indicator solutions. Typical transects were selected and soil samples taken from the various successional zones. From a number of transects two series of results

TABLE VI

CHEMICAL ANALYSES OF SOILS FROM LITTLE MOUNTAIN AREA; SALTS CALCULATED ON BASIS OF DRY WEIGHT OF SAMPLES. COLLECTED JULY 20, 1929. THE FIRST THREE WERE SAND, THE REMAINDER CLAY

LOCATION	WATER CONTENT	pH	NaCl	Na_2CO_3	$NaHCO_3$	Na_2SO_4	TOTAL
Barren salt flat 25 feet from plants	20.84	8.9	17.68	0.339	Trace	4.572	22.59
Two feet from first Salicornia plants........	15.94	8.8	5.89	0.005	0.05	0.852	6.72
Salicornia rubra pioneers.......	15.81	8.8	5.30	0.005	0.05	0.401	6.26
Suaeda erecta zone	14.80	9.8	2.60	0.005	0.672	Trace	3.31
Distichlis zone...	8.22	8.8	2.21	0.053	0.141	Trace	2.60
Cressa depressa zone..........	8.2	9.0	0.651	0.005	0.605	Trace	1.30

are presented here, illustrating the extreme alkali conditions and the average conditions respectively.

LITTLE MOUNTAIN AREA.—The strand at this point shows remarkable plant zonation, although the type of soil changes abruptly. The outer margin toward the lake is black sand and harbors pioneer samphires and a few annual inkweeds. The soil then changes to a heavy clay which bakes hard in late summer. In this part the inkweeds and salt grass are abundant, with scattered *Atriplex hastata*. Farther removed is a zone of *Cressa depressa* and some patches of *Sida hederacea*. *Salicornia rubra* at the margin of invasion is very short and becomes larger as the salt content of the soil decreases. Table VI shows the water, salt content, and pH of the soil.

The high salt content in these zones shows a unique extreme in salt tolerance and was the highest found in the region. KEARNEY,

BRIGGS, and SHANTZ (7) carried on investigations in Tooele Valley and report the average salt content of the *Salicornia* zone at 2.5%. HALKET (6) demonstrated that some species of *Salicornia* tolerate salt up to 5% in artificial cultures. The strand at this point has a high water table and the soils are saturated until summer sets in and then they begin to dry out slowly. The abundant water serves to dilute the strong salt and may be the main factor permitting the plants to survive in this highly saline habitat.

EASTERN SECTION.—The relatively flat beaches are of fine gray sand for the most part, with local areas of coarse calcareous material and decaying algal masses. Invading plants form an irregular line toward the lake side and seem to be limited only by high salt content of the soil and to a less extent by the mucky algal masses. The samphires are the most frequent plants to enter the barren areas, although the annual inkweed and salt grass lead the invasion in many places. Several transects of soil samples were taken through typical situations and the water and alkali conditions studied. The results bear out the conclusion that the critical period determining the vegetation is early spring when the water table is high and the strong soil solutes are more dilute than later in the season. The optimum salt concentration for the samphires ranges between 2.5 and 3.5% during the early spring, but conditions become variable as the water withdraws. Table VII shows the analyses of a typical area taken in July after the water table had gone down considerably.

The H-ion concentration of the soils throughout the region seems to show little or no relationship to the types of vegetation and in no way was it possible to use this as a factor determining the species or vegetation.

FLORISTICS.—The floristic features may be divided according to the predominating soil and topographic features, and in addition, the climatic and surface water relations. The eastern section receives about 10 inches more rainfall than the northern and western portions.

The eastern section, extending from Little Mountain to the Jordan delta, presents a strand of diverse situations. Numerous strand swamps, inlets, and springs introduce contrasting plant communities, while the greatest extent of the strand shows the usual pioneers al-

ready cited. The soil is of the black sand type previously mentioned. A bluff or terrace has been cut in the old delta deposits of the Weber River so that there is an abrupt break in the vegetation. This topographic feature narrows the successional zones so that there are no gradual transitional zones between the pioneer and intermediate types. To the north and south the terrace dwindles and the extremities show the gradual entrance of species from the saline plains.

The section extending from the Jordan delta to the southern tip of the lake is likewise one of diversity, with strand swamps, springs, calcareous dunes, and rocky headlands. The flora in this section

TABLE VII

ANALYSES OF SOIL SAMPLES FROM SALTAIR AREA; SALTS CALCULATED TO DRY WEIGHT OF SOIL. COLLECTED JULY 12, 1929

LOCATION	WATER CONTENT	pH	NaCl	Na_2CO_3	$NaHCO_3$	Na_2SO_4	TOTAL
Invading Salicornia rubra......	14.10	8.4	4.08	0.052	0.022	0.853	4.99
Suaeda erecta zone surface soil....	9.14	8.8	2.68	0.084	Trace	1.421	4.80
Same, 2 feet deep.	18.20	8.4	1.51	0.084	Trace	0.433	2.01
Distichlis zone surface soil....	3.59	8.6	0.07	0.057	Trace	0.497	0.62
Same, 2 feet deep.	10.81	8.6	0.06	0.10	0.012	0.384	0.54

sums up suitably most of the features common to the entire eastern side of the lake. The sand is very fine along the water's edge but toward the outer margin it becomes coarser, with calcareous material predominating. The invading species grow mainly in this coarser soil but a few may be noted in isolated colonies on the sand flats.

Generally there are several well defined zones. The pioneer types are more or less constant but the zones further removed become more and more diverse. This becomes evident in passing from one situation to another; the same pioneers persist but those following change or form different combinations with other species. Notable among the sandy beach elements are *Spartina*, *Abronia*, *Eriocoma*, and *Sphaerostigma*. The succession on the Saltair and Garfield beaches shows the following zones:

Zone 1. Salicornia rubra A. Nels.
S. utahensis Tidest.

Zone 2. Suaeda erecta (Wats.) A. Nels.
Zone 3. Distichlis spicata (L.) Greene
Zone 4. Spartina gracilis Trin.
Eriocoma cuspidata Nutt.
Puccinellia nuttalliana (Schultes) Hitchc.
Bromus tectorum L.
Abronia salsa Rydb.
Sphaerostigma utahensis Small
Zone 5. Sporobolus asperifolius (Nees. & Mey.) Thurb.
Distichlis spicata (L.) Greene
Atriplex hastata L.

Zones 4 and 5 are the most variable. Frequently *Suaeda moquinii* is present. The following plants are also common entrants:

Sporobolus airoides Torr.
S. cryptandrus (Torr.) Gray
Poa nevadensis Vasey
Bromus tectorum L.
Sitanion hystrix (Nutt.) J. G. Smith
Atriplex hastata L.
A. confertifolia (Torr.) Wats.
A. rosea L.
Pachylophus marginatus (Nutt.) Rydb.
Gutierrezia microcephala Gray
Chrysothamnus pulcherrimus Greene
Iva axillaris Pursh

Most of these plants are common inhabitants of the calcareous dunes forming the outer limits of the strand.

BURMISTER-DELLE CREEK SECTION.—This section is at the southern extremity of the lake and presents one of the broadest stretches of exposed beaches. At times when the lake is low the Stansbury Bar is exposed throughout the year, and only during the spring months are the extensive flats saturated with water. This is mainly precipitation and ground water. The general aspect presents a barren salt flat, white on the surface but with the underlying strata yellowish and with gray clays laminated with darker streaks. It retains the water until late summer when the outer margins become dry and cracked.

The topography north of Burmister and on the Stansbury Bar is interesting, as the salt flats are interrupted by a number of small island-like raised areas which stand 4–6 feet above the general level. They are composed of clay loam and harbor a flora of greasewood and shadscale which extends to the very margin and produces a marked contrast with the barren salt flats. These islands vary in size up to several hundred yards across.

For the most part there is no vegetation on the flats. Toward the mainland a few outposts of *Allenrolfea* and occasionally *Salicornia* appear in scattered colonies. *Allenrolfea* is almost the exclusive pioneer plant in many places. It builds the little hummocks described previously, often giving the surface a spotted appearance. The salt grass is generally the next plant to enter, with its characteristic habit of sending out long root stocks. A variety of plants may enter next, depending upon the locality and the conditions. The eastern portion finds a rich development of *Salicornia utahensis*, especially along moist playas where springs occur. Most of the portions surrounding the islands have species of *Suaeda* entering next, while toward the western portion little playa meadows are common. These may be occupied almost exclusively by salt grass or a mixed flora of salt grass, *Sporobolus airoides*, *S. cryptandrus*, *Puccinellia nuttalliana*, *Triglochin maritima*, and *Cressa erecta*.

The succession is various, often narrow in its zonation, and difficult to follow. The following arrangement indicates the usual order of approach:

Zone 1. Allenrolfea occidentalis (Wats.) Kuntze
Suaeda erecta (Wats.) A. Nels.
S. moquinii (Torr.) A. Nels.
Zone 3. Distichlis spicata (L.) Greene
Zone 4. Suaeda diffusa Wats.
Atriplex confertifolia (Torr.) Wats.
Sarcobatus vermiculatus (Hook.) Torr.

STANSBURY ISLAND.—The strand of Stansbury Island is continuous with that of the mainland at the present time. On either side at the south end it is very broad but it becomes gradually narrowed toward the middle of the island. On the east side and at the north it is

narrow, and often steep and rocky. In general the flora is much the same as that already described. The main contrasting feature is the presence of two parallel beach bars along the eastern shore near the middle. They are composed of a coarse, clean white calcareous sand arising from a shore of friable hardpan in process of genesis. The vegetation is in distinct zones with a number of new entrants. Notable among the latter are *Mentzelia*, *Cleome*, and *Amelanchier mormonica*. The steep slopes immediately behind these bars make it possible for a number of non-halophytic plants to make a rather close approach to the lake. The following zones may be recognized:

Zone 1. Salicornia rubra A. Nels.
S. utahensis Tidest.
Zone 2. Suaeda erecta (Wats.) A. Nels.
Allenrolfea occidentalis (Wats.) Kuntze
Zone 3. Atriplex argentea Nutt.
Amaranthus graecizans L.
Salsola pestifer A. Nels.
Mentzelia laevicaulis (Dougl.) T. & G.
Sphaerostigma utahensis Small
Zone 4. Distichlis spicata (L.) Greene
Bromus tectorum L.
Cleome serrulata Pursh
Amelanchier mormonica C.K. Schneider

In this vicinity a number of dead stumps of *Sarcobatus* and *Tetradymia spinosa* were observed, suggesting that the succession at one time reached a somewhat advanced stage before the rise of the lake killed them. In some places there is a well defined line of empty shells of the salt fly larvae, indicating the upper limits of the storm waves.

WESTERN AND NORTHERN SECTIONS.—The broadest expanse of barren strand is to be found in the western and northern portions of the lake. In many places the water's edge is nearly 2 miles from the old shore line. The white salt flats are interrupted only by drift wood and occasional hummocks of *Allenrolfea*. The latter were found nearly a mile out on the flats, with no intervening vegetation. In the major features the plants merely accentuate the richness and

halophytic character of the vegetation. The species show no further features beyond those already considered, and only local variations distinguish the region from the successional zones of the eastern and southern sections. The topography presents a very irregular line marking the old shore; long narrow terraces extend toward the lake with deep sinuous bays separating them. In the latter, salt grass meadows are common while pure strands of the various species may be found. Rich growths of *Suaeda*, *Salicornia*, *Allenrolfea*, and the several species of *Sporobolus* show these plants at their best.

The main influences of climate lie in light, heat, and evaporation. The bright glaring sunlight is perhaps more apparent than real and does not compare with that of high mountain tops, but the vegetation receives a maximum amount for valley regions. The surface temperature of the soil often reaches 36° to 38° C. The evaporation is high, and although the water supply is ample during the spring and early summer months, the gradual withdrawal of the ground-water, together with the increasing concentration of the soil solution, renders the strand one of the most unique of ecological situations.

INLETS, STRAND SWAMPS, AND SPRINGS

Fresh-water inlets and springs are common along the eastern side of the lake. At their points of entry into the lake the concentration of the salt is greatly reduced and a number of contrasting situations are to be found.

BEAR RIVER BAY.—The northern and eastern portions of Bear River Bay are bordered by broad mud flats, while the western border is more abrupt with several rocky points and numerous mineral springs on the strand. The Southern Pacific Railroad trestle extends across the mouth of the bay and forms a barrier which tends to retain the fresh-water in the bay and exclude the strong brines from the main body of the lake. The reduction in amount of salt from the trestle northward is as follows:

Little Mountain just north of the Southern Pacific trestle	18.01%
Willard Spur, south side	11.68%
Willard Spur, north side	8.60%

WILLARD SPUR AREA.—The Willard Spur is a shallow arm of Bear River Bay which extends eastward. The mud flats and substratum of the shallow water are undulating, with numerous slight depressions which retain little pools as the water recedes. Flaky accumulations of decaying vegetable matter lie half suspended in the water, while countless small crustaceans (*Cypris* sp.) crowd the margins and are left stranded as the water recedes. In the shallow pools a form of *Cladophora fraeta* occurs, often becoming very abundant. *Potamogeton pectinatus* and *Ruppia maritima* are other

TABLE VIII

PERCENTAGE MOISTURE, PH, AND PERCENTAGE TOTAL SALTS OF SOILS FROM WILLARD SPUR (SALTS ON BASIS OF DRY WEIGHT OF SAMPLES). COLLECTED JUNE 29, 1929

LOCATION	WATER CONTENT	PH	TOTAL SALTS
Clay silt from bottom of shallow pool	31.6	8.4	2.01
Clay flat, 20 feet from water	22.0	8.4	1.83
Dry clay flat, Salicornia wilting	10.2	8.6	1.76
Clay cracked and dry, Salicornia dead	8.0	8.8	1.31
Clay loam bordering cultivated fields	21.7	8.6	1.35
Same with Suaeda erecta	14.0	8.6	0.91

members of the aquatic society. *Salicornia rubra* germinates and reaches a robust size in water 2–3 inches deep. On the mud flats it is abundant as long as the water remains, but as summer passes desiccation becomes more and more severe until there is no longer sufficient water to maintain vegetation. The plants follow the water as it recedes. A transect of soil samples was taken from the water's margin for a mile back to the old shore line. Table VIII shows the analyses.

Lack of circulation of the water in the shallow portions of bays permits the accumulation of heat. Thus on July 29, with the air temperature at 32° C., the water of the main body of the lake was 19° C. at midday while the shallow water of Willard Spur was 31° C.

The mud flats are inhabited almost exclusively by *Salicornia rubra*. Following are a few scattered *Allenrolfea*, then an irregular zone of *Atriplex hastata*, and next an irregular zone of *A. truncata*. Grasses

enter next with numerous annuals and a few shrubs, forming a mixed association as follows:

Hordeum jubatum L.
H. gussonianum Parl.
Polygonum aviculare L.
Sarcobatus vermiculatus (Hook.) Torr.
Lepidium perfoliatum L.
L. pubicarpum A. Nels.
Sisymbrium altissimum L.
Allocarya nitens Greene
Matricaria suaveolens (Pursh) Buchen.
Grindelia squarrosa (Pursh) Dunal
Helianthus annuus L.

In seepage areas the following appear:

Puccinellia nuttalliana (Schultes) Hitchc.
Hordeum jubatum L.
Atriplex hastata L.
Spergularia salina Presl.
Ranunculus eremogenes Greene
Halerpestes cymbalaria (Pursh) Greene

THE PROMONTORY.—The strand on the east side of the Promontory is narrow, the mountains rising rather abruptly from the shore. A few steep headlands are conjunctive with the strand and the beach is strewn with rocks, in some places forming a shingle beach. The residual rocks are near the surface and numerous springs arise through the crevices, some bearing minerals and flammable gases.

The following algae were recorded:

Oscillatoria animalis Ag.
O. spp.
Enteromorpha intestinalis L.
Vaucheria sp.
Spirogyra sp.

The steepness of the strand in many places makes the zones of transition between the strongly saline soils and fresh soils very narrow, and consequently the successional zones are much contracted

and overlapped. The following zones are generalized from several situations:

Zone 1. Salicornia rubra A. Nels.
Zone 2. Polypogon monspeliensis (L.) Desv.
Distichlis spicata (L.) Greene
Zone 3. Hordeum jubatum L.
Halerpestes cymbalaria (Pursh) Greene
Zone 4. Scirpus americanus Pers.
S. paludosus A. Nels.
S. olneyi Gray

Meadows and muddy banks show a variety of plants and three main groups may be identified according to the degree of halophytism. Where the salt is strong the following occur:

Puccinellia nuttalliana (Schultes) Hitchc.
Distichlis spicata (L). Greene
Polypogon monspeliensis (L.) Desv.
Hordeum jubatum L.
H. gussonianum Parl.
Sitanion sp.
Juncus balticus Willd.
Rumex crispus L.
Suaeda erecta (Wats.) A. Nels.
Atriplex rosea L.
A. argentea Nutt.
A. hastata L.

Other situations show many composites:

Triglochin maritima L.
Sporobolus airoides Torr.
Spergularia salina Presl.
Melilotus alba Desv.
Achillea millefolium L.
Aplopappus lanceolatus (Hook.) T. & G.
Agoseris spp.
Helianthus annuus L.
Iva axillaris Pursh
Leontodon taraxacum L.

Some seepage areas have leached much of the alkali from the soil and present the following:

Lemna minor L.
Carex sp.
Berula erecta (Huds.) Coville
Glaux maritima L.
Asclepias speciosa Torr.
Castilleja exilis A. Nels.
Sonchus asper (L.) Hill

On the upper dry side of some of these mildly saline situations *Cleome serrulata* grows in dense masses; *Euphorbia glyptosperma*, *Polygonum aviculare*, *Artemisia tridentata*, and several species of *Chrysothamnus* are also found.

EASTERN SECTION.—From Little Mountain to East Bay there are numerous inlets and strand swamps. They are much alike in character and composition with a few variations according to the situation. *Cladophora* and *Spirogyra* are common; *Scirpus americanus* and *S. paludosus* usually appear in the inlets; the grasses *Puccinellia*, *Hordeum jubatum*, *Polypogon*, and *Distichlis* are common. Near the village of Hooper is a shallow strand swamp harboring a low and rather uniform vegetation. It is characterized by many small water channels with tussocks of plants and decaying accumulations. The algae are abundant; *Potamogeton pectinatus* and *Ruppia maritima* are submerged types and *Lemna minor* floats on the surface. The emergent society is composed of:

Sagittaria cuneata Scheld.
Scirpus spp.
Eleocharis palustris (L.) H. & B.
E. acicularis (L.) R. & S.
Cicuta occidentalis Greene
Berula erecta (Huds.) Coville

On the borders and on heaps of decaying vegetable matter the following enter:

Polypogon monspeliensis (L.) Desv.
Puccinellia nuttalliana (Schultes) Hitchc.
Sporobolus airoides Torr.

Atriplex hastata L.
Trifolium pratense L.
Epilobium adenocaulon Hausskn.

The swamp community in East Bay is one of contrasts. The outer portion toward the lake is much more saline than the portion toward the shore. The salt content of the water was found to be 5.86 and 1.03% respectively. The algae are abundant, appearing at a distance like a green meadow. *Salicornia rubra* is prominent and grows in water 2 or 3 inches deep. *Scirpus* spp. are present while *Typha* enters as a new member. The associated species include those already mentioned and also *Scirpus lacustris*, *Ranunculus eremogenes*, *Halerpestes cymbalaria*, and *Radicula nasturtium-aquaticum*.

Numerous other strand situations occur in other sections surrounding the lake, but present only slight variations of those cited. Black Rock, Lake Point, Delle Creek, and near the Salt Wells are other localities where fresh water enters.

River deltas

As physiographic units the deltas of the three largest rivers tributary to the lake are much the same in the character of the sediments deposited and in their vegetation. All are composed of heavy dark brown or gray clays of varying alkali content. The rivers are muddy. At present the conditions are much altered by the interference of duck clubs, dikes having been built around the distal extremities of the Jordan delta and across the north limb of the Bear River delta. Headgates in the dikes are closed during part of the year and the water backed up in the delta, extending the marsh land. Headgates have also been built at the point where the rivers enter the deltas and the flow of water through the various channels regulated. In the Jordan delta 3600 acres are submerged to a depth of 3 feet. Every spring the headgates are opened and the deltas drained. Strong winds often blow the lake brine back into the deltas, killing numerous fish and other animals besides increasing the alkali content of the soils.

The soil and water conditions when the Jordan delta was drained are shown in table IX.

The vegetation is augmented by introduction of seeds and cuttings of plants beneficial to ducks and other birds. Among the plants introduced are:

Sparganium eurycarpum Engelm.
Potamogeton pectinatus L.
Ruppia maritima L.
Scirpus americanus Pers.
S. validus Vahl.
S. lacustris L.
S. paludosus A. Nels.
Puccinellia nuttalliana (Schultes) Hitchc.

The seeds of these plants are relished very much by birds, and extensive fields of *Scirpus americanus* are cultivated. When the

TABLE IX

CHEMICAL ANALYSIS OF SOILS FROM JORDAN DELTA; SALTS CALCULATED IN TERMS OF DRY WEIGHT OF SAMPLES. COLLECTED JULY 29, 1929

LOCATION	WATER CONTENT	pH	NaCl	Na_2CO_3	$NaHCO_3$	Na_2SO_4	TOTAL SALTS
Salicornia.......	26.8	8.6	0.323	0.084	Trace	1.56	2.08
Scirpus spp......	22.8	8.6	0.389	0.084	"	0.99	1.40
Typha and Sparganium.......	25.8	8.6	0.328	0.082	"	0.81	1.27

deltas are drained in the spring the mud flats dry out and by August the fields are mowed. The following societies constitute the dominant flora:

Free-floating:

Spirogyra sp.
Cladophora fraeta forma

Anchored submerged:

Potamogeton pectinatus L.
Ruppia maritima L.

Emergent types:

Scirpus americanus Pers.
S. paludosus A. Nels.

S. lacustris L.
S. validus Vahl.
S. olneyi Gray

Emergent types restricted to muddy banks:

Sparganium eurycarpum Engelm.
Typha latifolia L.
T. angustifolia L.
Phragmites communis Trin.
Puccinellia nuttalliana (Schultes) Hitchc.
Distichlis spicata (L.) Greene
Rumex crispus L.
R. britannica L.
Salicornia rubra A. Nels.
Sonchus asper (L.) Hill

Types restricted to banks:

Triglochin maritima L.
Spartina gracilis Trin.
Hordeum jubatum L.
Polypogon monspeliensis (L.) Desv.
Atriplex hastata L.
A. rosea L.
Suaeda erecta (Wats.) A. Nels.
S. moquinii (Torr.) A. Nels.
Amaranthus retroflexus L.
Sida hederacea (Dougl.) Torr.
Asclepias speciosa Torr.
A. mexicana Cav.
Apocynum cannabinum L.
Iva axillaris Pursh
I. xanthifolia Nutt.
Grindelia squarrosa (Pursh) Dunal
Xanthium pennsylvanicum Wallr.
Helenium autumnale L.
Lactuca scariola L.
L. integrata (Gren. & Godr.) A. Nels.

Halerpestes cymbalaria becomes prominent along the margins after the delta is drained, while *Salicornia* invades the barren flats.

Playas and alkaline plains

A large part of the valleys surrounding Great Salt Lake have soils that are strongly alkaline. Successively these valleys have been the bed of the lake and the strands, and at present they have been reclaimed to a large extent by vegetation. The general physiographic features show a plain interrupted by playas, sloughs, drainage canals, and streams. Permanent streams traverse the region mainly on the eastern side, and it is on this side that much of the land has been turned to agricultural uses wherever drainage has been possible.

SOIL, ALKALI, AND GROUND-WATER.—Coarse grained oolitic sands and hardpan derivatives are common along the lake borders. The surface soil of the plains is dominantly Jordan loam with a subsoil of loam or clay. In the depressions the surface soil is usually Jordan clay with a subsoil of the same character. Sand is not frequent and hardpan is a subsoil type.

In a given column of soil the alkali content is often governed by the amount of water in it. In regions where the water table is deep the precipitation water tends to carry the salt deeper into the soil, leaving the surface layers with relatively small amounts. On the other hand, where the water table is close to the surface the water is drawn to the surface, carrying with it the soluble salts which are deposited in the upper layers of soil. A number of surveys of the soil and water conditions (1, 5, 7, 10, 11) furnish data which show that the percentage of alkali in the soil increases roughly in proportion to the shallowness of the water table, as follows:

Depth of water table below surface	Percentage of alkali
3 feet or less...........	3% or more
6 feet.................	1–3%
10 feet................	0.25–1%

Specific data correlated with the vegetation will be given in the following sections.

Playas

Playas are numerous and occur in nearly all parts of the valleys. Generally they are alike in soil, alkali, and water relations, few showing marked differences from the general conditions exhibited by the majority. Floristically they are strikingly similar although local variations are common. A distinction must be drawn for a correct understanding of this presentation. For convenience they may be considered in three groups: (1) the young undeveloped situations usually presenting a barren flat with a pioneer vegetation encroaching from the margins; (2) the intermediate situations where the entire playa has been invaded; (3) those which have been reclaimed by a halophytic flora. Most playas are alike in the early stages but diverse in the intermediate stages. None was observed in the mature stage.

TABLE X

GENERALIZED SOIL TRANSECT OF PIONEER PLAYA SHOWING PERCENTAGE ALKALI CALCULATED IN TERMS OF DRY WEIGHT OF SOIL. WATER TABLE THREE FEET OR LESS IN EACH INSTANCE. JUNE 25, 1929

No.	Type of plant	Surface	2 feet	3 feet
41	Barren area	7.06	6.55	10.91
42	" "	3.55	6.60	5.30
43	" "	3.02	5.05	5.00
54	Salicornia rubra	2.42	....	....
44	" "	2.00	4.15	4.11
35	Allenrolfea	0.98	1.00	1.03
36	"	1.54	1.61	1.60
45	Suaeda erecta	1.47	2.03	1.98
46	" "	1.48	1.68	1.70

Pioneer stages.—Pioneer types of the playas are much the same as those of the strand. The main difference lies in the habit and distribution of the plants. *Salicornia rubra* and *S. utahensis* are the most frequent species invading the barren playas, although *Allenrolfea occidentalis*, *Suaeda erecta*, *S. moquinii*, *S. occidentalis*, and *Distichlis spicata* are not uncommon in this capacity also. There seems to be little reaction of the plants to the type of soil in which they grow. The great determinative factor is a certain amount of

water; the great unifying factor is a certain amount of alkali. The amount of salt present in dry soils varies greatly, ranging from 0.05 to 10.0%. The concentration of soil solute depends upon the amount

TABLE XI

DOMINANT HALOPHYTES AND THEIR pH RELATIONS

No.	Type of plant	Locality	Type of soil	pH
109	Barren	Jordan Valley	Jordan clay	8.0
112		“ “	“ “	8.6
138		Weber Valley	“ “	8.9
139	Salicornia	Weber Valley	Salt Lake sand	9.0
108		Jordan Valley	Jordan loam	8.6
115		“ “	“ “	8.7
121		“ “	Salt Lake sand	8.4
169		Tooele Valley	Hardpan	8.4
170		“ “	Jordan clay	8.5
110	Suaeda erecta	Jordan Valley	Jordan clay	8.6
125		“ “	Salt Lake sand	8.4
186		“ “	Jordan loam	8.4
151		Weber Valley	Salt Lake sand	9.8
140		“ “	“ “ “	9.8
125	Suaeda moquinii	Jordan Valley	Salt Lake sand	8.4
132		“ “	Jordan loam	8.6
152		Weber Valley	Salt Lake sand	9.8
106	Allenrolfea	Jordan Valley	Jordan clay	9.0
110		“ “	“ “	8.6
111		“ “	“ “	8.2
187		“ “	Jordan loam	8.5
189		“ “	“ “	8.6
145		Weber Valley	Jordan clay	8.6
146		“ “	“ “	9.0
159		Tooele Valley	“ “	8.8
162		“ “	“ “	9.1
105	Distichlis	Jordan Valley	Jordan clay	8.8
181		“ “	Salt Lake sand	9.1
185		“ “	Jordan loam	8.4
193		“ “	“ “	8.6
200		Jordan delta	Jordan clay	8.6
153		Weber Valley	Salt Lake sand	9.6

of water in the soil, which is an extremely variable factor during the growing season. Soils of low percentage of salts and a small amount of water become as drastic as soils with high salt percentage and a large amount of water. Thus the strand has high percentage of salt and the water table is slow to withdraw, and *Salicornia* thrives in

these conditions. On the other hand the playas lose water faster and the soil solutes concentrate faster, so that the plants can persist only in soils showing approximately 2% salt in terms of dry weight. The values obtained from soil analyses of typical playa successions show the series to be very similar. The *Salicornia* belt averages about 2% salt, *Suaeda erecta* about 1.5%, and *Allenrolfea* about 1%. None of these plants was found in extremely strong salt as was the case on the strand. Table X represents the average conditions found in typical playas.

No correlation could be made between the H-ion concentration of the soil and the type of plants growing in it, nor could any correlation be made between the H-ion values and the types of soils and their water content. With such a range of values little can be interpreted other than the fact that these plants will tolerate a rather wide range. Table XI gives specific data in this connection.

INTERMEDIATE STAGES.—In the following discussions the term "intermediate stage" covers a number of diverse situations. In most playas there is generally an increase in the amount of alkali from the borders toward the center, so that the succession of plants follows as fast as the alkali is reduced. In some playas this is apparently not the case, however, but the alkali content is more or less uniform in all parts. In the first instance the vegetation is mixed and in the latter it is more or less homogeneous.

MIXED PLAYA SUCCESSION.—Following the pioneer species are two rather well defined belts of plants, the first group dominated by the Chenopodiaceae and the second by a number of small annuals. In group one the first three species named make up the vernal aspect and the remainder the summer aspect.

Triglochin maritima L.
Bromus tectorum L.
Puccinellia nuttalliana (Schultes) Hitchc.
Atriplex argentea L.
A. hastata L.
Suaeda intermedia Wats.
S. moquinii (Torr.) A. Nels.
Salsola pestifer A. Nels.

Bassia hyssopifolia (Parl.) Kuntze
Sesuvium sessile Pers.

The second group following these plants includes:

Deschampsia danthonioides Monro.
Myosurus apetalus Gay
Lepidium perfoliatum L.
L. dictyotum Gray
Hutchinsia procumbens (L.) Desv.
Arabis spp.
Allocarya nitens Greene
Plantago elongata Pursh
P. purshii Roem. & Schult.
Psilocarphus globiferus Nutt.

Of this second group all are annuals except *Deschampsia danthonioides*, and compose the vernal aspect. The alkali content of the soil of the first belt averages about 1.0% and of the second belt from 0.6 to 0.8%.

The terraces bordering the playas harbor a greasewood and shadscale association and certain elements of this association are creeping out on the playas. Among the plants noted are:

Atriplex confertifolia (Torr.) Wats.
A. nuttallii Wats.
Kochia vestita (Wats.) A. Nels.
Eurotia lanata (Pursh) Moq.
Sarcobatus vermiculatus (Hook.) Torr.
Tetradymia spinosa Gray

Reclaimed homogeneous playas.—It is assumed that a playa is reclaimed when it is occupied by plants in such numbers as to create sharp competition among them. In many instances a single species may invade a new area so as completely to occupy the ground and preclude the entrance of any other species except an occasional plant here and there. Uniform conditions partly explain this situation, but the more apparent reason is that the one species got there first in great numbers. Where playas are reclaimed by a single species there is usually a robust growth showing the plant at its optimum.

Soil samples were taken from selected communities of this type and the salt contents are shown in table XII.

SALICORNIA FLATS.—There are a few instances where a playa becomes completely invaded by dense growths of *Salicornia*. Usually in such areas the salt is rather strong but does not exceed the optimum of about 2.5 to 3%. The water table is usually high during a greater part of the year. One large community of this kind occurs in Hansel Valley.

TABLE XII

PERCENTAGE OF ALKALI IN SOILS OF RECLAIMED PLAYAS CALCULATED IN TERMS OF DRY WEIGHT. WATER TABLE 3 FEET OR LESS FROM SURFACE IN EACH INSTANCE. JUNE 28, 1929

No.	Locality	Surface	2 feet	3 feet
32	Allenrolfea flat	1.41	1.12	1.16
35	" "	0.98	1.00	1.03
36	" "	1.54	1.61	1.60
34	Suaeda moquinii flat	0.81	0.80	0.74
25	" " "	1.03	0.95	0.99
31	Distichlis meadow	0.96	1.01	1.12
33	" "	0.96	1.45	1.22
34	" "	0.82	0.84	1.00
29	Sporobolus field	0.55	0.89	1.07
30	" "	0.54	0.83	0.97

ALLENROLFEA FLATS.—This plant prefers a more open distribution, and while it becomes dense several other species are scattered throughout the flat. The alkali optimum is from 1.0 to 1.4%. Hansel and Curlew valleys show numerous communities of this type. The commonest species occurring with it are *Salicornia rubra* and *Suaeda erecta*. *Distichlis* also occurs with it where the water table is high. Generally the pickleweed tends to stay more or less by itself.

SALT GRASS MEADOWS.—It is not unusual to see continuous areas of dense salt grass. Of all the halophytic species in this region it is the most cosmopolitan. It occurs in nearly every kind of situation that furnishes a fair supply of water during the spring and early summer months. As a competitor in successions it tends to eliminate most plants, and once established it is slow to yield to other vegetation. Its success lies mainly in its habit of sending out long root stocks with numerous individuals disposed in straight lines. Its range of alkali tolerance is perhaps wider than that of any other

species. It grows in mildly saline soil and will also enter the most drastic soils following the samphires. With its relatively high competitive power it will dominate the ground wherever it can.

Where salt grass is dominant there are few and usually scattered associates. The most frequent ones are *Allenrolfea*, *Atriplex*, and *Suaeda*. The following is a complete list:

Atriplex hastata L.
A. argentea L.
Suaeda intermedia Wats.
S. occidentalis (Wats.) A. Nels.
Sida hederacea (Dougl.) Torr.
Glaux maritima L.
Cressa erecta Rydb.
C. depressa Good.
Adenostegia ramosa (Nutt.) Greene

The seasonal aspect is almost entirely summer and fall.

SPOROBOLUS FIELDS.—*S. airoides* grows in coarse caespitose bunches where the alkali content of the soil is about 0.5%. It is noted as a ground-water feeder. Associated with it are *S. cryptandrus*, *S. asperifolius*, and *Bromus tectorum*, besides a great many other less common plants. While its sociability is marked its habit of growth and soil relations are such that it does not afford a great deal of competition. The coverage is rather uniform where it is dominant, presenting dense tufts of feathery character but usually rather widely disposed. Interspersed is a wide variety of species. Of all the reclaimed playa situations the *Sporobolus* fields are the most variable and represent the commonest type preceding the greasewood-shadscale association.

With the exception of the species of *Sporobolus* and *Sitanion*, most of the vernal flora is composed of annuals included in the Graminaceae and Cruciferae. The composition is as follows:

Dominant species:

Sporobolus airoides Torr.
S. cryptandrus (Torr.) Gray
S. asperifolius (Nees. & Mey.) Thurb.
Bromus tectorum L.

Less common species:

Bromus hordeaceus L.
Hordeum gussonianum Parl.
Sitanion hystrix (Nutt.) J. G. Smith
Lepidium perfoliatum L.
L. dictyotum Gray
Malcolmia africana (Willd.) A. Br.
Draba nemorosa L.
D. micrantha Nutt.
Sophia pinnata (Walt.) Brit.
S. sophia L.
Sisymbrium altissimum L.
Camelina microcarpa Andrz.

In certain advanced stages in the reclamation of playas the vernal flora is followed by summer and autumn aspects represented almost exclusively by the Chenopodiaceae and Compositae. Notable among these are species of *Atriplex* and *Chrysothamnus*. The following is a more complete list, all of which are perennials:

Atriplex confertifolia (Torr.) Wats.
A. truncata (Torr.) Gray
A. nuttallii Wats.
Eurotia lanata (Pursh) Moq.
Kochia americana Wats.
K. vestita (Wats.) A. Nels.
Sarcobatus vermiculatus (Hook.) Torr.
Suaeda depressa (Pursh) Wats.
S. intermedia Wats.
S. moquinii (Torr.) A. Nels.
Gutierrezia microcephala Gray
Chrysothamnus nauseosus (Pall.) Brit.
C. pulcherrimus Greene
C. pinifolius Greene
C. viscidiflorus tortifolius (Gray) Greene
Viguiera ciliata (Robins. & Greene) Blake
Artemisia spinescens Eat.
A. tridentata Nutt.

GREAT AMERICAN DESERT.—This is a large playa 50 by 100 miles in extent and was the last extensive basin vacated by Great Salt Lake in its decline. The saline constituents permeate the soils to considerable depth and in many places the salt is relatively pure. The surface for the greatest extent is white with a crust of salt covering the yellowish and gray clays. The broad expanse is subject to wind erosion and the removal of vast amounts of salt during storms. While a large part of the desert is barren and without even an occasional plant to interrupt the monotony of the white surface, the area as a whole is far from being entirely barren. The pickleweed has become established in widely scattered hummocks along the broad border and in certain places in mid-desert. The samphires are scanty. Toward the surrounding hills the pickleweed increases in density, scanty growths of salt grass appear, and finally the inkweeds enter. The distribution of these plants is spotty and irregular, some places showing dense stands, but the greatest extent shows a poor, dwarfed, and wind-swept vegetation. The dual effects of climate and alkali render this area one of the most forbidding deserts on this continent, and the vegetation leading to its reclamation must meet a double problem.

Alkaline plains

The plains differ from the playas in being higher areas that do not accumulate water during the wet periods and that are otherwise well drained. For the greatest extent the soil is Jordan loam with a clay subsoil. In some areas hardpan is present as the subsoil. A few places are sandy. The water table is seldom less than 3 feet deep and in most areas 6 feet or more from the surface. Saline conditions cover a wide range and their consideration will be left for specific citation.

FLORISTICS.—The flora is diverse, often mixed, and in most regions forming certain well defined combinations that are rather constant over wide areas. Some of these combinations may be considered as associations while others less extensive and less well defined will be considered simply as plant communities. The following are recognized: greasewood-shadscale association (*Sarcobatus vermiculatus* and *Atriplex confertifolia*), shadscale association, *Kochia* flat association (*K. vestita* and *K. americana*), *Kochia-Suaeda* communities, *Sarcobatus-Grayia* communities, and rabbit-brush communities (*Chrysothamnus* spp.)

GREASEWOOD-SHADSCALE ASSOCIATION.—This is the most extensive association of the region and is second only to *Artemisia tridentata* as a basin type of vegetation. It grows in all quarters of the region, borders most parts of the Great American Desert, and is

TABLE XIII

PHYSICAL COMPOSITION OF SOILS OF GREASEWOOD-SHADSCALE ASSOCIATION IN PERCENTAGE OF PARTICLES OF DIFFERENT SIZES IN TERMS OF DRY WEIGHT AND H-ION CONCENTRATION

No.	Locality	Soil	Diameter of particles (mm.)						pH
			6	5	3	2	1	½	
166	Tooele Valley	Loam	3.5	3.0	4.3	6.1	6.5	76.6	9.4
162	Tooele Valley	Clay	0.0	0.0	0.0	0.4	1.4	98.2	9.1
107	Jordan Valley	Loam	0.0	0.2	0.2	0.2	3.5	95.9	8.6
134	Farmington	Loam	0.4	1.3	2.8	11.8	26.2	57.5	8.9

TABLE XIV

WATER AND TOTAL SALTS CALCULATED IN PERCENTAGES OF DRY WEIGHT OF SOILS FROM GREASEWOOD-SHADSCALE ASSOCIATION

No.	Locality	Soil and subsoil	Water table (ft.)	Total salts (%) at		
				1 in.	2 ft.	3 ft.
24	Jordan Valley	Jordan loam Jordan clay	3	0.83	0.82	0.83
37	" "	Jordan loam Jordan clay	3	0.75	0.82	0.82
38	" "	Jordan loam hardpan	6	0.21	0.22	0.18
39	" "	Jordan loam hardpan	6	0.26	0.18	0.18

found generally in most saline situations throughout the west. It encroaches on both pioneer and intermediate floras of the strand and playas. The general aspect presents a scrubby vegetation 3–5 feet in depth with alternating patches of the greasewood and shadscale, the greasewood being the most extensive member. This plant has a much branched stem with white shreddy bark and green wood, and numerous spiny branches with terete fleshy leaves. The roots penetrate the soil to a depth of 10–15 feet and the plant is noted as a

ground-water indicator. It is also an indicator of black alkali, sodium carbonate.

The shadscale is a round-topped bush acquiring a height of 3 feet in its robust forms. The leaves are broad and thick with a silvery coat of scurfy scales. The physical factors governing this vegetation center around the alkali content of the soil and to a less extent the depth of the water table. Soil samples were taken from many localities, most of which show the conditions to be very similar. Tables XIII and XIV show the typical ranges of conditions.

The composition of this association varies in different localities, the areas approaching the lake showing a predominance of the Chenopodiaceae while the distal margins where the alkali is reduced show an abundance of grasses and annuals. The composition is as follows:

Dominant shrubs:

Atriplex confertifolia (Torr.) Wats.
A. truncata (Torr.) Gray
A. nuttallii Wats.
Sarcobatus vermiculatus (Hook.) Torr.
Eurotia lanata (Pursh) Moq.
Kochia vestita (Wats.) A. Nels.
Suaeda moquinii (Torr.) A. Nels.
Chrysothamnus nauseosus (Pall.) Brit.
Tetradymia spinosa H. & A.

Frequent herbs:

Sclerochloa dura (L.) Beauv.
Aristida longiseta Steud.
Sitanion hystrix (Nutt.) J. G. Smith
Atriplex argentea L.
A. hastata L.
A. rosea L.
Amaranthus graecizans L.
Cleome lutea Hook.
C. serrulata Pursh
Opuntia fragilis (Nutt.) Haw.
O. polyacantha K. Sch.

Cressa erecta Rydb.
Grindelia squarrosa (Pursh) Dunal
Ambrosia psilostachya DC.
Machaeranthera leucanthemifolia Greene
Lactuca integrata (Gren. & Godr.) A. Nels.
L. scariola L.

These plants include principally the perennials and such annuals as persist until late summer. The vernal aspect is remarkable in its number and density of short-lived annuals. The most dominant members include *Bromus hordeaceus*, *B. tectorum*, *Hordeum gussonianum*, and many species of the Cruciferae. In the early spring the flats are green but by the end of June these plants form a carpet of parched, tawny brown herbage. In various sections of the greasewood-shadscale areas the density is different so that the predominance of the vernal types is variable. In dense stands of the shrubs the vernal plants are sometimes very scanty, while in areas where the shrubs are widely disposed the vernal growths are ample and rather suggest a shrubby grass flat community. The composition of the vernal flora is as follows:

Dominant species:

Bromus tectorum L.
B. hordeaceus L.
Hordeum gussonianum Parl.
Lepidium perfoliatum L.
Erodium cicutarium L'Her.

Subdominant species:

Lepidium dictyotum Gray
Hutchinsia procumbens (L.) Desv.
Capsella bursa-pastoris Medic.
Malcolmia africana (Willd.) A. Br.
Sophia pinnata (Walt.) Brit.
S. sophia L.
Camelina microcarpa Andrz.
Alyssum alyssoides (L.) Gouan.
Draba nemorosa L.
D. micrantha Nutt.

Thelypodium sagittatum (Nutt.) Endl.
Sisymbrium altissimum L.
S. asperum DC.
S. repandum L.
Lomatium platycarpum (Torr.) C. & R.
Aulospermum longipes (Wats.) C. & R.
Lappula redowskii occidentalis (Wats.) Rydb.
Castilleja linariaefolia Benth.
Adenostegia sp.
Plantago elongata Pursh
P. purshii Roem. & Schult.
Matricaria suaveolens (Pursh) Buchanan

Mosses:

Pterygoneurum cavifolium (Ehrh.) Jur.
Tortula ruralis Ehrh.
T. subulata inermis B. & S.
Pottia nevadense Card. & Ther.
Funaria hygrometrica (L.) Sibth.
F. calcarea Wahl.
Bryum caespiticium L.
B. inclinatum (Sw.) Bland.
B. pendulum (Hornsh.) Schimp.

The variety of plants is much greater on the east side of the lake than on the west side, since the east side receives about 10 inches more annual rainfall than the western part. The valleys along the southern and western sections of the lake show the association at its best. In Curlew and Hansel valleys on the northwestern borders the plants are not so robust, but present a monotonous combination of green and silvery gray for miles. The water table is remote except along the immediate lake borders. The annuals are fewer and the grasses less prominent. The dominant plants are more evenly balanced, the notable ones being as follows:

Common species:

Atriplex truncata (Torr.) Gray
A. nuttallii Wats.
A. saccaria Wats.

A. falcata (Jones) Standl.
Grayia spinosa (Hook.) Moq.
Suaeda moquinii (Torr.) A. Nels.
S. intermedia Wats.
S. diffusa Wats.
Kochia vestita (Wats.) A. Nels.
Eurotia lanata (Pursh) Moq.

Frequent species:

Eriocoma cuspidata Nutt.
Sitanion hystrix (Nutt.) J. G. Smith
Eriogonum pusillum T. & G.
Opuntia sp.
Gutierrezia microcephala Gray
Chrysothamnus nauseosus (Pall.) Brit.
Artemisia tridentata Nutt.
A. spinescens Eat.

A remarkable stand of greasewood and shadscale is found bordering the old lake shore in the Lakeside Mountain region. In most places it has the normal aspect as to depth of vegetation and density, but there is one tract which shows a relatively pure stand of large greasewoods which attain a height of 10 feet. The composition is somewhat varied by a greater abundance of *Tetradymia*, *Grayia*, and *Opuntia*.

The greasewood-shadscale association is the edaphic climax vegetation of the Great Salt Lake region. At its outer limits it is invaded mainly by *Artemisia tridentata* and by a mixed grass-shrub community of plants that is not very well defined. It is not a permanent vegetation but one of long duration. The guarantee of its persistence is alkali. Physical forces are continually but slowly reducing the salt content of the soil. The chief agencies are wind and water. The former removes vast amounts during dust storms and deposits non-saline dust from other regions; the latter tends to carry the salt in solution to the lake and deposit non-saline detritus from the hills above. The reaction of the plants to the reduction of alkali is a critical point which may be of short or long duration, depending upon conditions. Where halophytes are partially covered by flood deposits

they may persist, but new progeny are forced out by the non-saline types. The broad plains are slow in giving up the alkali, and only near the outer borders is the ingress of non-halophytes creating sharp and critical competition. The halophytes lose ground mainly through lack of progeny to maintain the hold established when saline conditions were nearer the optimum. Upsetting the equilibrium which guarantees the persistence of the halophyte reduces its relative competitive power, and thus it is forced closer and closer to the center of the alkali. It is another phase in the dynamics of vegetation which covers long periods of time. With the slow procession crowding the halophytes ever closer to the lake, and with the possible extinction of the lake, they are destined to occupy a much smaller area than they now hold. Successions in remote regions may arise and culminate, perhaps perish, before the lowest point on the Great Salt Lake basin is reclaimed by a non-halophytic vegetation climax. Whether this will ever occur can be left only to conjecture. Diastrophism may cut it short, or a change in climate may refill Great Salt Lake.

SHADSCALE ASSOCIATION.—Extensive stands of the shadscale frequently occur on the distal margins of the greasewood-shadscale associations. The general aspect presents a low shrubby vegetation, but the plants are usually rather widely disposed. It is essentially a flora of dry, mildly saline soils. Abundant ground water is necessary to support greasewood and many other halophytes, but *Kochia*, *Eurotia*, and many species of *Atriplex* have the capacity to grow in dry soils. High well drained ground and particularly long alluvial slopes are favorable for development of the shadscale association. Regions of this character are found in Tooele Valley, the valleys bordering the Great American Desert, and in Curlew Valley. *Atriplex confertifolia* is the dominant shrub. The vernal flora is much like that described in the preceding association. The composition of the summer aspect is as follows:

Shrubs:

Atriplex confertifolia (Torr.) Wats.
A. nuttallii Wats.
A. truncata (Torr.) Gray
Eurotia lanata (Pursh) Moq.
Kochia vestita (Wats.) A. Nels.

Tetradymia spinosa H. & A.
T. nuttallii T. & G.
Artemisia spinescens Eat.
Chrysothamnus pumilus Nutt.
C. nauseosus (Pall.) Brit.
Gutierrezia microcephala Gray

Herbs:

Poa sandbergii Vasey
Festuca octoflora Walt.
Bromus tectorum L.
Agropyron tenerum Vasey
Eriogonum cernuum Nutt.
E. sp.
Salsola pestifer A. Nels.
Amaranthus graecizans L.
Lepidium perfoliatum L.
Draba nemorosa L.
D. micrantha Nutt.
Arabis holboellii Hornem.
Malcolmia africana (Willd.) R. Br.
Cleome lutea Hook.
Astragalus utahensis T. & G.
Sphaeralcea grossularieafolia (H. & A.) Rydb.
Opuntia sp.
Chrysopsis foliosa Nutt.
Aster sp.
Antennaria sp.

KOCHIA FLAT ASSOCIATION.—*Kochia* is a small shrub seldom more than 1 foot high with a more or less herbaceous upper stem and a woody base. The leaves are narrow, slightly fleshy, and covered with long soft hairs. There are two species in this region, *K. vestita* and *K. americana*. The general aspect of the association is a low shrub of grayish color and relatively few associates. The ground is usually exposed more than in most associations and communities. The soil relations are shown in table XV.

Kochia is not a particularly deep rooting plant and typically feeds

on precipitation water. In nearly all places where it occurs as an association the flats are dry and well drained. Tooele, Skull, and Curlew valleys show the association at its best. Most of the associated

TABLE XV

ANALYSIS OF SOILS OF KOCHIA FLAT ASSOCIATION. SALTS IN TERMS OF DRY WEIGHT OF SOIL

No.	Locality	Soil subsoil	pH	Total salts (%) at		
				1 in.	2 ft.	3 ft.
17	Skull Valley	Jordan clay	8.4	1.62	1.64	....
17a	Skull Valley	Jordan clay	8.4	1.03	1.16	1.30
26	Jordan Valley	Jordan loam hardpan	8.2	0.26	0.27	0.26
64	Tooele Valley	Jordan loam Jordan clay	8.6	0.84	0.86	0.86

plants are the same as previously listed but occur in different degrees of abundance. The following are the commonest members:

Shrubs:

Kochia vestita (Wats.) A. Nels.
K. americana Wats.
Eurotia lanata (Pursh) Moq.
Atriplex truncata (Torr.) Wats.
A. nuttallii Wats.
Tetradymia glabrata Gray
T. spinosa H. & A.

Herbs:

Poa sandbergii Vasey
Deschampsia danthonioides Munr.
Bromus tectorum L.
Lepidium perfoliatum L.
Plantago purshii Roem. & Schult.

KOCHIA-EUROTIA COMMUNITIES.—This community is merely a rather well defined variation of the *Kochia* flat association, differing mainly in having *Eurotia* in equal dominance with *Kochia*. The community is generally denser than the preceding association but the species are about the same.

KOCHIA-SUAEDA COMMUNITIES.—This is another variation of the *Kochia* flat association, occurring in local areas of dry and rather strongly saline soil. The general aspect is darker than the preceding community, with *Suaeda depressa*, *S. diffusa*, and an occasional greasewood forming the principal members. Other species are few and widely scattered.

RABBIT-BRUSH COMMUNITIES.—As one of the most prominent plants of the lowlands, the rabbit-brush finds an important rôle in many associations and by itself often constitutes the dominant aspect in certain localities. It is essentially a ground water indicator and thrives in mildly saline soils. Its limits of tolerance vary with the type of alkali present. In the Great Salt Lake region where chlorides and carbonates are strong its range appears to be between 0.1 to 0.3%, although in local places it has been observed to grow in soils bearing as high as 0.8% salt. The Tooele Valley study (7) reports an average of 0.3%, while in other regions in the state it has been reported in soils of 3.15% salt (8). When high concentrations are found gypsum is usually the principal salt.

The most extensive rabbit-brush communities are located in Tooele and Blue Spring valleys. Frequently it is associated with greasewood or *Sporobolus*. On Stansbury Island it commonly grows with the greasewood on the flats and with the sage on the higher levels. It is one of the dominant plants of sand dunes. Scattered growths occur in nearly all parts of the region along the junction between alkaline and non-alkaline soils.

The general aspect presents a community of somewhat open growth with caespitose erect stems 3–4 feet high, the individuals rather evenly disposed. Grasses and small herbs usually occupy the intervening spaces. The composition is as follows:

Principal species:

Chrysothamnus pinifolius Greene
C. viscidiflorus tortifolius (Gray) Greene
C. nauseosus (Pall.) Brit.
C. puberulus (Eat.) Greene
C. graveolens (Nutt.) Greene
C. pulcherrimus Greene

Sporobolus airoides (Torr.) Gray
Distichlis spicata (L.) Greene

Frequent species:

Bromus tectorum L.
Allium acuminatum Hook.
Zygadenus paniculatus Wats.
Comandra pallida DC.
Kochia vestita (Wats.) A. Nels.
Atriplex hastata L.
A. confertifolia (Torr.) Wats.
Eurotia lanata (Pursh) Moq.
Sarcobatus vermiculatus (Hook.) Torr.
Suaeda moquinii (Torr.) A. Nels.
Lepidium perfoliatum L.
Camelina microcarpa Andrz.
Sophia sophia L.
Malcolmia africana (Willd.) R. Br.
Erodium cicutarium L'Her.
Opuntia fragilis (Nutt.) Haw.
Grindelia squarrosa (Pursh) Dunal
Aster leucanthemifolius Greene
Artemisia tridentata Nutt.
A. gnaphalodes Nutt.
Cirsium scariosum Nutt.
Lactuca integrata (Gren. & Godr.) A. Nels.

Dunes

The dunes of Great Salt Lake are formed of two kinds of sand, calcareous oolitic sand and fine-grained siliceous sand. Considerable stress was laid on the formation of calcareous deposits through the precipitation of calcium carbonate from the lake waters in the earlier part of this paper. All of the dunes bordering the lake are of the calcareous type while those removed from the lake are of the siliceous type.

Saltair section.—Extending from Saltair to the Jordan Delta is a series of low dunes of calcareous character. The grains are coarse and often mixed with a stony element derived from hardpan. The

finer material shows more than 98% particles less than 0.5 mm. in diameter by dry weight, while the coarser material averages a little over 50% particles of this size. The alkali content is shown in table XVI.

The general aspect presents the light colored oolitic and hardpan material dotted with shrubs and tufts of grass while a variety of small herbs are scattered between. The greasewood and rabbit-brush are the dominant shrubs and *Eriocoma* and *Sporobolus* the dominant

TABLE XVI

WATER AND CHEMICAL ANALYSIS OF OOLITIC DUNE DEPOSIT OF SALTAIR SECTION. SALTS CALCULATED IN TERMS OF DRY WEIGHT OF SAMPLES

Depth	Water content	NaCl	$NaCO_3$	$NaHCO_3$	Na_2SO_4	Total salts
4 in.	4.59	0.16	0.057	Trace	0.497	0.62
2 ft.	12.81	0.255	0.100	0.01	0.284	0.64

herbs. In local depressional areas the salt grass, samphires, and ink-weeds become prominent. The older dunes show a mixed plant community with both halophytic and non-halophytic plants growing together with no apparent diminution in vigor or robustness. The composition is as follows:

Shrubs:

Atriplex confertifolia (Torr.) Wats.
A. canescens (Pursh) James
A. truncata (Torr.) Gray
Sarcobatus vermiculatus (Hook.) Torr.
Gutierrezia microcephala Gray
Chrysothamnus pulcherrimus Greene

Frequent herbs:

Eriocoma cuspidata Nutt.
Sporobolus airoides Torr.
Distichlis spicata (L.) Greene
Bromus tectorum L.
Poa sp.
P. sandbergii Vasey

Sitanion hystrix (Nutt.) J. G. Smith
Atriplex hastata L.
Salsola pestifer A. Nels.
Abronia salsa Rydb.
Sphaerostigma utahensis Small
Chaenactis stevioides H. & A.

STANSBURY ISLAND DUNES.—On the west side of the island extending from the middle to the northern extremity is a series of dunes. It varies in width up to nearly 300 yards and varies in depth up to 15 or 18 feet. The sand is of the oolitic calcareous type, white on the surface, but at 2 or 3 feet in depth it is of finer texture and brownish in color. There is also a siliceous element at this depth. At the northern end the light material has been blown high up on the rocky hillside. Presumably the deposits rest on a level saline plain.

The floral aspect is dominated by two plant types. The southern part shows a scattered shrub community dominated by greasewood and rabbit-brush while toward the north *Juniperus utahensis* offers an interesting and unique situation. Many typical dune species are to be noted in *Eriocoma*, *Eriogonum*, *Abronia*, and the Onagraceae. The moss *Tortula ruralis* may be properly classed as a dominant member, great tufts occurring frequently under shrubs and on the north-facing slopes.

The shrubby areas may be traced by the prominent white color of *Chrysothamnus puberulus*, which is robust in habit and lends a distinct character to the vegetation as a whole. The composition of the shrubby community is as follows:

Dominant shrubs:

Sarcobatus vermiculatus (Hook.) Torr.
Atriplex canescens (Pursh) James
Chrysothamnus puberulus (Eat.) Greene

Frequent shrubs:

Grayia spinosa (Hook.) Moq.
Chrysothamnus lanceolatus Nutt.
C. stenophyllus (Gray) Greene
C. viscidiflorus (Hook.) Nutt.
C. viscidiflorus tortifolius (Gray) Greene

Frequent herbs:

Eriocoma cuspidata Nutt.
Sporobolus cryptandrus (Torr.) Gray
Bromus tectorum L.
Agropyrum tenerum Vasey
Elymus condensatus Presl.
Comandra pallida DC.
Eriogonum cernuum Nutt.
E. ovalifolium Nutt.
Salsola pestifer A. Nels.
Abronia fragrans Nutt.
A. micrantha Torr.
Erysimum sp.
Astragalus utahensis T. & G.
Mentzelia acuminata (Rydb.) Tidest.
M. laevicaulis (Dougl.) T. & G.
Pachylophus sp.
Sphaerostigma utahensis Small
Ptiloria exigua (Nutt.) Greene
Chylisma parryi (Wats.) Small
Leptodactylon pungens (Torr.) Wats.

The depressions are more alkaline, as indicated by the presence of *Distichlis* and *Sporobolus*. *Bryum* sp. is also found here.

The northern half of the dunes shows the entrance of the juniper, which is slowly invading the shrubby communities, the first entrants being small and shrubby. The species just listed persist, although fewer in numbers, as the junipers become more and more dominant. As the dunes become larger the trees increase in size and numbers in proportion. In addition to the plants cited, the juniper community has the following:

Juniperus utahensis (Engelm.) Sarg.
Hordeum pusillum Nutt.
H. maritimum With.
Chenopodium album L.
Atriplex rosea L.
Erodium cicutarium L'Her.
Rhus trilobata Nutt.

The presence of the juniper is a point of special interest in that it marks the closest approach of trees to the lake. Its tolerance of mild concentrations of alkali is well known in other parts of the state, where it reaches its maximum development.

LAKESIDE MOUNTAIN DUNES.—One of the most interesting dune areas is that paralleling the base of the Lakeside Mountains, about 2 miles from the old lake shore. They are mainly siliceous and of a very fine tan-colored sand. While their origin is obscure, they may have been calcareous dunes at one time and have since had the calcium washed away, leaving the siliceous nuclei of the original oolite.

Sarcobatus and *Atriplex canescens* are the commonest shrubs. The open places show many characteristic species, among which are *Eriocoma*, *Eriogonum cernuum*, *Rumex venosus*, *Abronia salsa*, *A. cycloptera*, a species of *Psoralea*, and *Chamaesyce flagelliformis*. The following list shows the composition:

Shrubs:

Atriplex canescens (Pursh) James
A. confertifolia (Torr.) Wats.
Grayia spinosa (Hook.) Moq.
Chrysothamnus viscidiflorus tortifolius (Gray) Greene
Artemisia tridentata Nutt.
Tetradymia spinosa H. & A.

Frequent herbs:

Eriocoma cuspidata Nutt.
Sitanion hystrix (Nutt.) J. G. Smith
Eriogonum cernuum Nutt.
Rumex venosus Pursh
Chenopodium album L.
C. fremontii Wats.
C. sp.
Atriplex rosea L.
Amaranthus graecizans L.
Abronia salsa Rydb.
A. cycloptera Gray
Erysimum sp.
Cleome lutea Hook.

Psoralea lanceolata Pursh (?)
Chamaesyce flagelliformis (Engelm.) Rydb.
Opuntia fragilis (Nutt.) Haw.
Oenothera sp.
Chylisma parryi (Wats.) Small
Leptodactylon pungens (Torr.) Nutt.
Cryptanthe sp.
Solanum triflorum Nutt.
Marrubium vulgare L.
Machaeranthera sp.
Ptiloria exigua (Nutt.) Greene
Helianthus annuus L.

Tortula ruralis is the only moss.

Salt marshes and sloughs

Salt marshes and sloughs may arise in a variety of ways. They occur in localities where the water table is very close to the surface, where fresh water or saline springs and wells have their outlets. Tooele, Jordan, and Malad valleys are the principal localities, while numerous smaller areas are to be found in other places.

Analyses of the soils from the margins and beds of marshes and sloughs show conditions to be much like those already cited. Toward the outer limits of the alkali belt all gradations of saline quality of the soil may be observed, from those strongly impregnated with alkali to those that are comparatively sweet. The H-ion concentration of both soil and water shows much the same reactions as noted before. Table XVII gives a list of localities and the pH concentrations. Analyses of the waters from the principal mineral springs are given in the section dealing with hot mineral springs.

BLACK ROCK-TOOELE VALLEY MARSHES.—Topography plays a rôle in some places. At Black Rock, springs are found along the base of a steep headland so that extremes in alkalinity are found within a very short range. Southward and entering Tooele Valley the marshes are established in the depressions. The floral aspects are much the same as in most marshes in this region, with rushes and cattails lending the usual dominating characteristics. The features of contrast are noted mainly in subsidiary types. In this instance the

algae are of particular interest, showing types that are adjusted to mildly saline water. Among the specimens collected are two species of *Enteromorpha* which are abundant in some places and *Spirogyra*

TABLE XVII

H-ION CONCENTRATION OF SOILS AND WATERS FROM VARIOUS SALT MARSHES, SLOUGHS, AND SPRINGS OF GREAT SALT LAKE REGION

No.	Type of vegetation	Locality	Soil or water	pH
157	Batrachospermum	Jordan Valley	Spring water	7.4
154	Radicula, Glaux, Glycyrrhiza	" "	Black loam	7.8
155	Scirpus olneyi	" "	" "	8.6
156	Sporobolus	" "	" "	7.6
105a	Cladophora, etc.	" "	Well water	7.6
105	Distichlis	" "	Slough clay	8.8
100	River water	Jordan River	Water	8.0
135	Distichlis	Farmington	Slough clay	8.5
188	Barren	Jordan Valley	Slough bed	8.4
189	Allenrolfea	" "	Slough margin	8.6
190	Suaeda occidentalis, Bryum, etc.	" "	" "	8.4
191	Allenrolfea	" "	" "	9.2
193	Distichlis	" "	" "	8.6
194	Chara, Typha, Scirpus	" "	Salt marsh	8.6
195	Distichlis, Sida, etc.	" "	Marl loam	8.4
196	Atriplex hastata	" "	" "	8.2
197	Scirpus, Aster angustus, etc.	" "	" "	8.2
199a	Myxophyceae	" "	Well water	8.3
202	Scirpus paludosus	" "	Slough bed	8.5
203	Distichlis, Suaeda	" "	" "	8.3
204	Myxophyceae	" "	Hot spring	8.4

and *Cladophora* which are abundant in other places. The algal flora is:

Myxophyceae:

Aphanothece nidulans Rich.
Tolypothrix tenuis Kz.

Chlorophyceae:

Chlamydomonas spp.
Cladophora spp.
Enteromorpha intestinalis L.
E. plumosa Kz.

Potamogeton pectinatus was the only submerged vascular plant found. The emergent members form dense and extensive societies. The rushes are most prevalent in the more strongly saline portions

of the marsh while cattails are restricted to the margins where the salt is not so concentrated. The following plants were listed:

Typha latifolia L.
T. angustifolia L.
Scirpus americanus Pers.
S. lacustris L.
S. olneyi Gray
S. paludosus A. Nels.
Eleocharis palustris (L.) R. & S.

The muddy banks generally show a mixture of true halophytes and plants of sweet soils. The grasses are usually dominant. This society includes:

Eleocharis acicularis (L.) R. & S.
Polypogon monspeliensis (L.) Desf.
Distichlis spicata (L.) Greene
Puccinellia nuttalliana (Schultes) Hitchc.
Juncus balticus Willd.
Carex nebraskensis Dewey
Rumex persicarioides L.
R. crispus L.
Atriplex hastata L.
Halerpestes cymbalaria (Pursh) Greene
Radicula nasturtium-aquaticum (L). Brit. & Rendle
Cicuta occidentalis Greene
Berula erecta (Huds.) Coville

Conjunctive with the marshes are meadow-like societies harboring many grasses and a wide variety of other plants. The components include:

Triglochin maritima L.
T. palustre L.
Sporobolus asperifolius (Nees & Mey.) Thurb.
S. airoides Torr.
S. confusus (Fourn.) Vasey
Polypogon monspeliensis (L.) Desf.
Spartina gracilis Trin.
Beckmannia erucaeformis (L.) Host.

Phragmites communis Trin.
Puccinellia nuttalliana (Schultes) Hitchc.
Hordeum jubatum L.
Bromus tectorum L.
Distichlis spicata (L.) Greene
Juncus balticus Willd.
Rumex persicarioides L.
R. crispus L.
R. britannica L.
Atriplex hastata L.
Bassia hyssopifolia (Pall.) Kuntze
Abronia salsa Rydb.
Myosurus apetalus Gay
Ranunculus eremogenes Greene
Halerpestes cymbalaria (Pursh) Greene
Spergularia rubra (L.) J. & C.
S. salina Presl.
Glaux maritima L.
Dodecatheon salinum A. Nels.
Gaura parviflora Dougl.
Onagra hookeri (Torr. & Gray) Small
Asclepias speciosa Torr.
Castilleja stricta Rydb.
Adenostegia ramosa (Nutt.) Greene
Iva axillaris Pursh
Helianthus annuus L.

The abrupt slopes of the headlands make a narrow conjunctive belt with the marshes and springy areas which is much reduced in alkali content so as to present a different community of plants:

Hordeum jubatum L.
Salix exigua Nutt.
Urtica breweri Wats.
Atriplex rosea L.
Sophia pinnata (Wats.) Brit.
Sisymbrium altissimum L.
Cleome serrulata Pursh

Rosa melina Greene
Melilotus alba Desv.
Trifolium repens L.
Glycyrrhiza lepidota Pursh
Rhus trilobata Nutt.
Onagra hookeri (T. & G.) Small
Berula erecta (Huds.) Coville
Asclepias speciosa Torr.
Cuscuta sp. on Helianthus et al.
Cressa erecta Rydb.
Marrubium vulgare L.
Castilleja linariaefolia Benth.
C. exilis A. Nels.
Rubia tinctoria L.
Grindelia squarrosa (Pursh) Dunal
Iva axillaris Pursh
Ambrosia psilostachya DC.
Xanthium pennsylvanicum Wallr.
Helianthus annuus L.
Helenium autumnale L.
Crepis runcinata T. & G.
Lactuca integrata (Gren. & Godr.) A. Nels.
L. scariola L.

Malad Valley and Blue Spring Hills section.—This region includes extensive marsh lands, many of which have been extended artificially for the purpose of maintaining them as bird sanctuaries. As in the case of the river deltas, several plants beneficial to birds have been introduced. *Ruppia*, *Potamogeton*, *Sparganium*, *Puccinellia*, and several rushes are the principal ones. In general these marshes are similar in plant types although they differ in minor respects. The following species were peculiar to this section:

Sparganium eurycarpum Engelm.
Lemna minor L.
Spirodela polyrhiza (L.) Schleich.
Potamogeton natans L.
Ruppia maritima L.

Echinochloa crus-galli (L.) Beauv.
Scirpus validus Vahl.
Carex stipata Willd.
Polygonum emersum (Michx.) Brit.
Epilobium adenocaulon Hausskn.
Helianthus nuttallii T. & G.

Sloughs

Sloughs occur chiefly in Jordan Valley. They differ from the marshes in being poorly drained, are generally more saline, and the grasses replace the rushes and cattails as the dominant types. There are numerous plants distributed in many families. The bottoms are muddy with flocculent masses of black muck consisting of finely divided vegetal matter mixed with clay. The algal flora of these situations is of particular interest. In general those areas strongly alkaline have a preponderance of Myxophyceae while localities less alkaline show a decided increase in Chlorophyceae. Wherever sloughs are maintained by wells or springs of mineral water Myxophyceae are also the dominating algae. The following is a composite list:

Myxophyceae:

Oscillatoria amphibia Ag.
O. animalis Ag.
O. amoena (Gom.) Kz.
O. chalybea Mert.
O. janthophora
O. nigro-viridis Thw.
O. subtilissima Kuetz.
O. spp.
Phormidium angustissimum W. & G. S. West
P. foveolarum (Gom.) Mont.
P. fragilis Menegh.
P. ramosum P. Boye
Aphanothece calthrata West
A. nidulans Rich.
Anabaena spp.
Lyngbya martensiana Menegh.

L. aeruginea-caerulea (Kuetz.) Gom.
Tolypothrix tenuis Kz.
Nostoc pruniforme (L.) Ag.
N. spp.

Chlorophyceae:

Ulothrix zonata (?)
U. spp.
Spirogyra spp. (five species indet.)
Zygnema spp.
Oedogonium spp.
Rhizoclonium hieroglyphicum Kz.
Nephrocystium agardhianium Nag.
Gloeocystopsis limneticus Am.
Cladophora fraeta forma
C. spp.
Enteromorpha intestinalis L.
E. plumosa Kz.

Rhodophyceae:

Batrachospermum spp.

Charaphyceae:

Chara foetida L.
C. fragilis ?

Several other species were included in the collections to which no definite names could be assigned. This was particularly true of the unicellular forms.

Among the vascular plants the grasses are the dominant types, the salt grass being almost exclusive in this respect in most localities. Most of the species listed for the marshes occur on the margins of adjacent areas. Besides the plants listed previously the following were noted for the sloughs alone:

Poa nevadensis Vasey
Elymus glaucus Buckley
E. triticoides Buckley
Juncus nevadensis Wats.
Polygonum ramosissimum Michx.

P. convolvulus L.
P. aviculare L.
Chenopodium album L.
C. glaucum L.
C. leptophyllum (Moq.) Nutt.
Atriplex falcata (Jones) Standl.
A. nitens
Suaeda depressa (Pursh) Wats.
S. intermedia Wats.
S. moquinii (Torr.) A. Nels.
Amaranthus blitoides Wats.
Sesuvium sessile Pers.
Lepidium perfoliatum L.
Sida hederacea (Dougl.) Torr.
Apocynum cannabinum L.
Asclepias incarnata L.
Cressa erecta Rydb.
Marrubium vulgare L.
Aster frondosus (Nutt.) T. & G.
A. angustus T. & G.

Hot mineral springs

Hot springs are found along the base of the Wasatch Mountains and at the south end of the Blue Spring Hills. The origin of these springs is believed to have been brought about through faulting in which a great amount of heat was generated by the friction. The great depth serves as insulation while the crushed rocks along the fault plane serve to collect percolating waters.

The temperature of the water varies from 34° to 61° C. While sulphurous in quality the dominant constituent is sodium chloride in every case. The H-ion concentration varies from 8.2 to 8.8. Analyses of the most important springs are given in table XVIII. The discharge from some of these springs is considerable, the largest two being Beck's Hot Spring and a spring near the town of Honeyville. The water wells up in great volume with a characteristic sulphurous odor.

The flora of the springs proper is exclusively of Myxophyceae

while in the outlets a limited number of Chlorophyceae and vascular plants occur. There is a rather marked uniformity in the species inhabiting the various springs, although the larger springs show the richest and most varied flora. *Oscillatoria amphibia* and *O. animalis*

TABLE XVIII

ANALYSES OF WATERS FROM HOT MINERAL SPRINGS OF GREAT SALT LAKE REGION, IN TERMS OF P.P.M., EXCEPT D WHICH IS PERCENTAGE (2)

	A	B	C	D	E	F	G
Ca	694.3	141.5	535.2	4.90	901.0	1174.0	878.0
Mg	109.5	27.7	138.4	0.4	218.0	28.0	379.0
Na	3754.9	405.0	3039.0	30.38	16595.0 (Na + K)	8563.0	10426.0
K	196.9	55.0	178.0	3.76			
Al_2O_3	9.0	....	....	0.02	....	....	....
Fe_2O_3	....	5.1	0.7	....	....	....	....
SiO_2	31.5	50.5	21.3	0.20	....	....	....
SO_4	840.5	53.8	787.5	0.94	497.0	203.0	20.0
HCO_3, CO_2	204.8	272.7	442.9	0.61	454.0	186.0	393.0
Cl	6743.8	635.6	4968.0	58.79	27081.0	15079.0	18460.0
Fe	....	....	....	....	1.0	1.0	2.0
Total	12584.9	1658.0	10284.0	23309.0	45541.0	25300.0	30440.0

A. Beck's Hot Spring, Temp. 133°F. BRIGGS, B. R., U.S.G.S. Bull. 42. 148. 1878.
B. Sandy Hot Springs, 8 miles south of Salt Lake City. KINGSBURY, J. T., 1882.
C. Warm Springs, Salt Lake City. KINGSBURY, J. T., 1881.
D. Utah Hot Springs, 8 miles north of Ogden. Temp. 55° C. CLARKE, F. W., U.S.G.S. Bull. 9. 30. 1884.
E. Hot Spring at Honeyville, Boxelder Co. BAILEY, J. R.
F. Sanitarium Hot Springs, Boxelder Co. BAILEY, J. R.
G. Hot Spring at south end of Little Mountain (north of Bear River Bay). BAILEY, J. R.

were found in every spring investigated. The following is a composite list of all the springs:

Oscillatoria amphibia Ag.
O. amoena (Gom.) Kuetz.
O. animalis Ag.
O. brevis var. neapolitana (Kuetz.) Gom.
O. chalybea Mert.
O. janthophora
O. laetevirens Crouan.
O. lemmermannii Wolosz.

O. nigro-viridis Thw.
O. subtilissima Kuetz.
O. tenuis Ag.
Phormidium autumnale (Ag.) Gom.
P. angustissimum W. & G. S. West
P. foveolarum (Mont.) Gom.
P. ramosum P. Boye
Spirulina calderia Tild.
Lyngbya martensiana Menegh.
L. aerugineo-caerulea (Kuetz.) Gom.
Synechococcus spp.

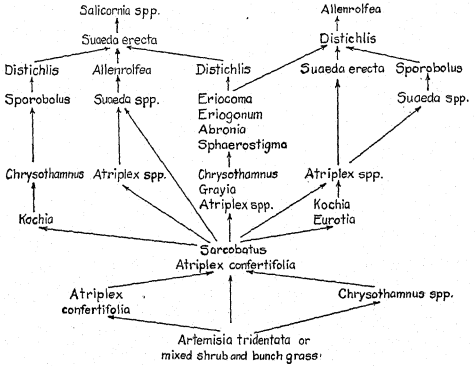

FIG. 1.—Diagram representing principal succession encroaching on strands and playas of Great Salt Lake.

Summary of successional aspects and floristics

There are two pioneer types of the halosere in this region, *Salicornia* and *Allenrolfea*. The former is most commonly followed by *Suaeda erecta* and the latter by *Distichlis*. It is of interest to note that *Salicornia* and *Allenrolfea* do not ordinarily grow together as invaders of the barren flats, but are not uncommonly associated some distance from the line of invasion. The plants making up the belts or zones succeeding are so various that it is difficult to select

any one or two types as representative of the region. *Distichlis*, *Sporobolus*, species of *Suaeda* and of *Atriplex* cover such a wide and diverse area that tracing a uniform line is almost impossible. They occur in nearly every combination as the intermediate successional stage. The diagram shown in figure 1 shows the actual successions of some of the larger and representative localities from various points around the lake.

The floristic features are tabulated in tables XIX and XX. In the first are listed the number of species in each family showing four relative degrees of halophytism. Column I includes all those that are absolute halophytes found only in alkaline soil; column II includes plants essentially halophytes but with a wide range of toleration; column III is a group of plants essentially non-halophytic but frequently inhabiting saline areas; column IV includes such plants as are seldom found in halophytic regions. According to this estimate, 54.3% of the flora is essentially halophytic and 45.7% essentially non-halophytic but with a certain degree of alkali tolerance.

Table XX indicates the geographic relations as to extent of distribution. The endemic species are those occurring only within a 100-mile radius of Great Salt Lake; the western species are those of the Rocky Mountain region and westward. A little over half of the flora is typically western.

Summary

1. The rocks of the Great Salt Lake region embrace a wide range of igneous, sedimentary, and metamorphic members. Historically they extend from the Archean to the Eocene, with nearly every period represented.

2. Great Salt Lake had its origin in a large Pleistocene lake named Lake Bonneville. It covered an area of 19,750 square miles and at its highest level stood about 1000 feet above the present level of Great Salt Lake. It was partially drained by erosion of the outlet and then by evaporation and seepage.

3. The climate shows an annual precipitation of about 6–7 inches in the western portion of the region and about 16–17 inches in the eastern portion. Evaporation amounts to about 68.67 inches a year, with an average wind movement of 69,139 miles yearly. The growing season free from frost averages approximately 160 days and the annual average temperature is about 50° F.

TABLE XIX

	I	II	III	IV	TOTAL
Pinaceae	....	....	1	....	1
Gnetaceae	....	....	2	....	2
Typhaceae	....	....	2	....	2
Sparganiaceae	....	....	1	....	1
Lemnaceae	....	....	....	1	1
Naiadaceae	....	2	1	1	4
Juncaginaceae	....	....	2	....	2
Alismaceae	....	....	2	....	2
Gramineae	7	14	12	5	38
Cyperaceae	2	7	4	....	13
Juncaceae	....	1	2	....	3
Liliaceae	....	....	....	3	3
Melanthaceae	....	....	1	....	1
Salicaceae	....	....	....	3	3
Urticaceae	....	....	2	....	2
Polygonaceae	....	3	7	3	13
Chenopodiaceae	22	8	....	....	30
Amaranthaceae	....	1	1	1	3
Nyctaginaceae	....	3	2	....	5
Aizoaceae	1	....	....	....	1
Caryophyllaceae	1	1	....	....	2
Ranunculaceae	1	1	3	1	6
Cruciferae	....	14	6	3	23
Capparidaceae	....	2	....	1	3
Rosaceae	....	....	....	1	1
Pomaceae	....	....	....	1	1
Leguminosae	....	3	6	1	10
Geraniaceae	....	....	1	....	1
Euphorbiaceae	....	1	2	....	3
Anacardiaceae	....	....	....	1	1
Malvaceae	1	3	....	....	4
Loasaceae	....	....	1	2	3
Cactaceae	....	2	2	....	4
Onagraceae	1	4	3	2	10
Haloragidaceae	....	....	....	1	1
Umbelliferae	....	1	5	2	8
Primulaceae	2	....	....	....	2
Apocynaceae	....	....	1	1	2
Asclepiadaceae	....	1	3	1	4
Cuscutaceae	....	....	....	....	1
Convolvulaceae	2	....	2	....	4
Polemoniaceae	....	2	1	3	6
Boraginaceae	2	3	....	1	6
Verbenaceae	....	....	1	1	2
Labiatae	....	1	....	1	2
Solanaceae	....	2	....	....	2
Scrophulariaceae	1	2	2	3	8
Orobanchaceae	....	....	1	....	1
Plantaginaceae	....	2	....	1	3
Rubiaceae	....	....	1	....	1
Compositae	1	42	16	12	71
Total	44	130	94	59	326
Percentage	13.5%	40.8%	27.9%	17.8%	....

TABLE XX

	Endemic	Western	North American	Cosmo-politan	Disjunct
Pinaceae	....	1	....	....	....
Gnetaceae	....	2	....	....	....
Typhaceae	....	....	....	2	....
Sparganiaceae	....	....	....	2	....
Lemnaceae	....	....	....	1	....
Naiadaceae	....	....	....	4	....
Juncaginaceae	....	....	....	2	....
Alismaceae	....	....	1	2	....
Gramineae	....	13	12	13	1
Cyperaceae	....	1	4	8	....
Juncaceae	....	....	1	2	....
Liliaceae	....	3	....	....	....
Melanthaceae	....	1	....	....	....
Salicaceae	....	....	3	....	....
Urticaceae	....	1	1	....	....
Polygonaceae	....	3	3	7	....
Chenopodiaceae	2	18	4	5	1
Amaranthaceae	....	1	....	2	....
Nyctaginaceae	....	4	1	....	....
Aizoaceae	....	1	....	....	....
Caryophyllaceae	....	1	1	....	....
Ranunculaceae	....	3	2	1	....
Cruciferae	....	8	4	10	1
Capparidaceae	....	....	2	1	....
Rosaceae	....	1	....	....	....
Pomaceae	1	....	....	....	....
Leguminosae	1	3	1	5	....
Geraniaceae	....	....	....	1	....
Euphorbiaceae	....	1	....	2	....
Anacardiaceae	....	1	....	....	....
Malvaceae	....	3	1	....	....
Loasaceae	....	3	....	....	....
Cactaceae	....	3	1	....	....
Onagraceae	1	8	....	1	....
Haloragidaceae	....	....	....	1	....
Umbelliferae	....	5	2	1	....
Primulaceae	....	1	....	1	....
Apocynaceae	....	....	....	2	....
Asclepiadaceae	....	3	2	....	....
Convolvulaceae	....	2	....	....	....
Polemoniaceae	....	6	....	....	....
Boraginaceae	....	6	....	....	....
Verbenaceae	....	....	1	1	....
Labiatae	....	....	2	....	....
Solanaceae	....	1	....	1	....
Scrophulariaceae	....	5	....	3	....
Orobanchaceae	....	1	....	....	....
Plantaginaceae	....	....	2	1	....
Rubiaceae	....	....	....	1	....
Compositae	4	45	9	13	....
Total	9	160	59	97	3
Percentage	2.4%	48.7%	18.1%	29.2%	0.6%

4. At present Great Salt Lake is about 75 miles long and 38 miles wide, with an average depth of 15 feet, the deepest point being about 40 feet. The brine consists principally of sodium chloride with smaller amounts of potassium, magnesium, and calcium salts. The percentage varies with the fluctuation of lake level, showing approximately 1% for every foot increase or decrease. At its highest level it showed 13.8% and at its lowest level 27.72%.

5. Floristically the region is characterized by the absence of trees, by broad barren salt flats bordered with succulent salt plants, and by a low shrubby vegetation on the saline plains.

6. The lake water proper harbors six species of algae, four Myxophyceae and two Chlorophyceae. *Aphanothece utahensis* and *Microcystis packardii* are the commonest species and are directly concerned with the formation of a tufa-like deposit which later forms a hardpan. *Chlamydomonas* sp. is abundant also.

7. The strand flora consists mainly of two invading genera, *Salicornia* and *Allenrolfea*. The former tolerates salt up to 6%, the commonest amounts being about 3% at the optimum. The latter averages between 1 and 1.5% at the optimum.

8. The strand succession is represented generally by the following zonation:

Zone 1. Salicornia rubra
S. utahensis
Allenrolfea occidentalis
Zone 2. Suaeda erecta
Zone 3. Distichlis spicata
Zone 4. Suaeda spp.
Zone 5. Sporobolus spp.
Atriplex spp.
Sarcobatus vermiculatus
Chrysothamnus spp.

9. Strand swamp types include *Scirpus americanus*, *S. paludosus*, *Potamogeton pectinatus*, *Ruppia maritima*, *Polypogon monspeliensis*, *Puccinellia nuttalliana*, and a variety of other plants.

10. The river deltas are modified by human agency and cultivation. Species of *Scirpus* predominate in most places while *Sparganium*, *Potamogeton*, *Typha*, and numerous salt marsh plants constitute a rich flora.

11. The strands of Great Salt Lake are bordered for their greatest extent by playas and alkaline plains with a few rocky headlands.

12. The soil is dominantly Jordan clay and Jordan loam with a clay or hardpan subsoil. The water table ranges from less than 3 feet to remote depths, but usually not far below 10 feet in most places.

13. The alkali relations are intimately connected with the water table. In general the percentage of alkali is roughly proportional to the depth of the permanent water, as follows:

Depth of the water table	Percentage alkali
3 feet or less	3% or more
6 feet	1–3%
10 feet	0.25–1%

The H-ion concentration of the soils ranges from pH 8.0 to 9.4, the general average being pH 8.4 to 8.6.

14. The range of alkali toleration by the principal pioneer plants is:

	Maximum	Optimum	Minimum
Salicornia	6%	2–3%	0.5–1%
Suaeda erecta	4%	1–2%	0.25%
Allenrolfea	3%	1–1.5%	0.25%
Distichlis	2%	0.7–1%	0.25%

15. The vegetation of the playa-plains region is dominated by low shrubs of silvery gray and dark green color and numerous small annuals. There are a few algae, lichens, and mosses but no liverworts nor pteridophytes. Trees are absent.

16. Playas are invaded by the following succession of plants: *Salicornia rubra*, *S. utahensis*, *Suaeda erecta*, *Allenrolfea occidentalis*, *Distichlis spicata*, numerous small herbs in the Graminaceae and Cruciferae, and finally *Sarcobatus vermiculatus* and species of *Atriplex*.

17. Playas may be reclaimed almost exclusively by a single species. Homogeneous situations may show *Salicornia* flats, *Allenrolfea* flats, *Distichlis* meadows, or *Sporobolus* fields. In contrast the playas may be reclaimed by a mixed vegetation.

18. The alkaline plains show an average range of 0.6 to 1% alkali in the soil.

19. The following associations are well defined: greasewood-

shadscale, *Sarcobatus vermiculatus-Atriplex confertifolia*, forming the dominant vegetation; shadscale association, *Atriplex confertifolia; Kochia* flat association. Less well defined are the following plant communities: *Kochia-Eurotia, Kochia-Suaeda, Sarcobatus-Grayia*, and *Chrysothamnus* spp.

20. Dunes show a characteristic vegetation of *Sarcobatus, Atriplex* spp., *Chrysothamnus* spp., and a number of herbaceous species typical of sandy situations.

21. Salt marshes and sloughs show numerous algae and aquatic plants typical of mildly saline water. *Scirpus* spp., *Typha, Distichlis, Juncus, Eleocharis*, and a variety of herbs are the principal plants.

22. Hot mineral springs carry salt up to 5% and vary in temperature up to 61° C. Myxophyceae are the only plants. *Oscillatoria* spp. are dominant.

Critical determinations of the vascular plants were made by Dr. A. O. GARRETT, Dr. P. A. RYDBERG, and Dr. F. S. BLAKE. Dr. A. J. GROUT checked some of the mosses and Dr. N. L. GARDNER named most of the algae. Thanks are due to Dr. H. C. COWLES and Dr. G. D. FULLER for their criticisms.

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SEROLOGICAL STUDIES OF PLANT VIRUSES

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Introduction

The discovery of viruses as supposedly different infectious agents has led to a problem of classification, the difficulty of which has become increasingly apparent.

Since the viruses could not be visually or culturally identified, they came to be characterized largely by what they did, that is, by the reactions they produced in the plant; and it became the custom to name the virus on the basis of the symptoms it produced on a single host (for example, tobacco mosaic, tomato mosaic, or cucumber mosaic). Confusion arose when later experiments showed: (1) that the symptoms produced by a given virus on a given plant vary with the source and mode of infection, the variety of the plant, its age, and the environment in which it has grown; (2) that the symptoms produced by a given virus may vary in different plants; and (3) that different viruses may produce similar symptoms on the same plant. Obviously a more reliable method of differentiation and classification of plant viruses is needed.

JOHNSON and HOGGAN (6) recommend that the present system be abandoned and an attempt be made to devise some system more comparable with the one used in classifying bacteria and fungi. Our knowledge, however, is as yet insufficient for classifying viruses, since present methods of study have yielded little positive information concerning their nature or characteristic properties which are of value in grouping them (10).

It has been shown that living things, plant and animal, by virtue of their chemical constituents have antigenic specificities which characterize the species, and that these antigenic specificities can be studied most advantageously by means of immunological or serological reactions. This approach has proved valuable in the study of the relationships, pathogenic properties, and chemical constituents of microorganisms (13), and might yield valuable information when applied to the classification of plant viruses.

The major difficulty in the use of these methods is that of securing a suitable preparation of the virus antigen. Since viruses cannot be grown in artificial mediums, it is difficult to secure antigens satisfactory in regard to purity and concentration. Such crude antigens as the extract of plant tissue contain antigenic substances other than the virus, which must be considered in interpreting the results. The few serological studies on plant viruses already made, however, although confined to the use of crude extracts and limited largely to the virus of tobacco mosaic, suggest that this method has possibilities.

Using the precipitin test, DVORAK (3) has shown that mosaic of potato affects the antigenic specificity of the globulin fraction of the cell juice and the cytoplasm. PURDY (8, 9) has shown that the juices from healthy plants and from plants affected with mosaic have antigenic substances in common. The juice of pathic tobacco plants contained an additional fraction which could not be removed from the homologous antiserum by absorption with healthy plant antigen. After such absorption, the anti-mosaic serum reacted with the juice (antigen) of tomato, petunia, pepper, and several species of tobacco, all of which had been infected with the virus of tobacco mosaic but not with the juice from healthy plants of these species. PURDY concludes from these results that the antigenic fraction which is present in the diseased plants and absent in healthy ones represents the virus, and not an altered host protein as was concluded by DVORAK. The work of MATSUMATO and SOMAWAZA (7) on tobacco mosaic substantiates PURDY's conclusions. These workers found that when the juice from tobacco and tomato plants affected with mosaic was digested with trypsin and used as a test antigen, reactions were not obtained with healthy plant antiserum but were obtained with mosaic plant antiserum. The tryptic digest was infectious, indicating that, although the healthy plant proteins were apparently broken down so far that they no longer acted as antigens, the virus had withstood the trypsin sufficiently to remain antigenic.

The characteristic antigenic property of a virus-diseased plant as reported by these workers might be due to (1) alteration by the virus of the normal healthy antigenic constituents of the plant, (2) linkage of the virus to the normal healthy constituents of the plant,

or (3) the virus itself. This paper deals with an attempt to determine which of the foregoing factors is responsible for the specificity of the serological reactions, and whether these reactions can be used in differentiating and classifying plant viruses.

Part I

Problem

An attempt was made to determine whether the characteristic specificity of the serological reactions with plant virus is due to alteration of the normal healthy antigenic constituents of diseased plants or to the virus itself.

It was thought that purification of the virus by methods which involved different principles and testing of these preparations by the precipitin technique might produce evidence on the specificity of the reaction for the virus. In order to prepare serologically pure antigens it is necessary to remove contaminating antigenic substances. Since the preponderance of evidence indicates that only proteins or protein complexes can function as true antigens, it follows that the removal of plant proteins is of paramount importance in preparing serologically pure virus antigens. The test for purity of virus antigens is the presence or absence of a precipitin reaction with the healthy plant antiserum. If plant proteins are present in the virus antigen, the homologous antiserum will react with the juice of healthy plants used as antigen. If plant protein is not present, no precipitin reaction will be obtained. However, if the purified virus is antigenic and a precipitin reaction is obtained only with preparations containing the virus, the reaction can be logically attributed to its presence.

Experimental procedure

First, antigens were prepared from healthy tobacco plants and from mosaic (Tobacco Virus I Johnson) plants using several methods of purification; second, rabbits were immunized with these antigens; and, third, the immune sera produced were used for the precipitin ring tests. Readings of these tests were made after one and two hours' incubation at 25° C., and were confirmed after the tubes had remained in the icebox overnight.

PREPARATION OF ANTIGENS.—The five antigens used in this experiment were: healthy plant antigen,[1] crude mosaic antigen, acetone precipitate antigen, tryptic digest antigen, and tomato mosaic antigen $Al(OH)_3$.

Young healthy tobacco plants were ground in a food chopper and the juice extracted from the resulting pulp by subjecting it to 5000 pounds' pressure. The juice was then centrifuged, and the liquid passed through a Berkefeld filter and used without further treatment as healthy plant antigen.

Juice from tobacco plants that had been inoculated with mosaic virus (Tobacco Virus I Johnson) was pressed out according to the method used in preparing healthy plant antigen, and divided into three portions which were treated individually to form three kinds of virus antigens. One portion was filtered through a Berkefeld V filter and used without further treatment as crude mosaic antigen. A second portion of 500 cc. of juice was treated with lead acetate and barium hydroxide to precipitate proteins, salts, and carbohydrates, then centrifuged, and the supernatant treated with two volumes of acetone. The acetone precipitate was filtered off and taken up in 50 cc. of distilled water. This suspension is hereafter referred to as acetone precipitate antigen. According to VINSON and PETRE (12) this method of purification gives high concentration of relatively pure virus. The third portion of the juice was digested for 48 hours with 2 per cent trypsin, passed through a Berkefeld V filter, and used as tryptic digest antigen. Coagulated egg white was added to one tube of the mosaic plant juice and trypsin to check the activity of the trypsin.

The method of BREWER, KRAYBILL, SAMSON, and GARDNER (2) was followed in preparing tomato mosaic antigen $Al(OH)_3$. Tomato plants which had been inoculated with Virus I Johnson were ground in a food chopper, and the juice strained through gauze and supercentrifuged at 33,000 r.p.m. The residue was taken up in water, shaken vigorously, centrifuged, and the supernatant cleared with $Al(OH)_3$. This suspension was highly infectious, as evidenced by the number of local lesions on *Nicotiana glutinosa* (4). It did not give a biuret test for protein.

[1] All plants were grown in the greenhouse.

Sodium chloride was not used to extract the ground plant tissue since there is a possibility that it might increase the plant proteins in the expressed juice.

PRODUCTION OF ANTISERA.—Rabbits were immunized in duplicate with the five antigens described. Intraperitoneal injections of 5 cc. each were given every third day. A test bleeding made after the fourth injection showed no reactions with the healthy plant antigen against its homologous serum. The rabbits were then given

TABLE I

PRECIPITIN TESTS WITH HEALTHY PLANT JUICES IN PRESENCE OF HOMOLOGOUS AND HETEROLOGOUS ANTISERA

?=INDECISIVE, o=NO REACTION, +=FAINT RING, ++=SMALL BUT DEFINITE RING, +++=MODERATE RING, ++++=WIDE RING

ANTISERUM	HEALTHY PLANT ANTIGEN DILUTIONS								CONTROL NACL
	1:1	1:2	1:4	1:8	1:16	1:32	1:64	1:128	
Healthy tobacco..	+++	+++	+++	++	+	o	o	o	o
Crude mosaic.....	+++	+++	+++	+++	++	+	o	o	o
Tryptic digest of mosaic plant...	?	?	?	o	o	o	o	o	o
Acetone precipitate of mosaic plant.	?	o	o	o	o	o	o	o	o
Tomato mosaic $Al(OH)_3$.......	o	o	o	o	o	o	o	o	o
Control (normal rabbit).........	o	o	o	o	o	o	o	o	o

three more injections of 5 cc. each, and on the sixth day after the last injection bled aseptically. The blood was allowed to clot and the immune sera collected in sterile bottles.

PRECIPITIN RING TESTS.—The sera were pipetted into small precipitin tubes and layered with the desired dilution of antigen. The tubes were incubated at 25° C. for an hour and were then examined for the presence of a ring of precipitate at the interphase between the serum and the antigen. These readings were checked after another hour's incubation. The tubes were placed in the icebox overnight and examined for precipitate the next morning. All tests were run in duplicate. Each antigen was tested with homologous and heterologous antisera. Table I illustrates the method of setting up such a test with one antigen.

DISCUSSION

The results of preliminary experiments with crude extracts of healthy and diseased plants indicated that these materials had antigenic substances in common. (The precipitin tests are summarized in table II.) This is what is to be expected, of course, since unaltered plant protein was probably present in both types of preparation and would constitute a common antigen.

TABLE II

PRECIPITIN TESTS WITH FIVE ANTIGENS AND THEIR HOMOLOGOUS AND HETEROLOGOUS ANTISERA

o = NO REACTION, + = DEFINITE PRECIPITATE

ANTISERUM (UNDILUTED)	ANTIGEN (DILUTION 1:4)						
	HEALTHY TOBACCO PLANT	CRUDE TOBACCO MOSAIC PLANT	ACETONE PRECIPITATE TOBACCO MOSAIC VIRUS	$Al(OH)_3$ PURIFIED TOMATO MOSAIC VIRUS	TRYPTIC DIGEST OF TOBACCO MOSAIC PLANT	CONTROL 2% TRYPSIN*	CONTROL NACL
Healthy plant..........	+	+	o	o	o	o	o
Crude tobacco mosaic....	+	+	+	+	+	o	o
Acetone precipitate tobacco mosaic virus.......	o	+	+	+	+	o	o
$Al(OH)_3$ purified tomato mosaic virus..........	o	+	+	+	+	o	o
Tryptic digest of tobacco mosaic plant..........	o	+	+	+	+	+	o
Normal rabbit serum (control)................	o	o	o	o	o	o	o

* Trypsin is antigenic and gives a precipitin test with tryptic digest antisera. However, this does not invalidate the results of the precipitin tests with other antigens.

Further experiments, summarized in table II, showed also that crude extracts of mosaic-diseased plants and purified preparations of virus from such extracts also had common antigenic factors. It might be expected that this would prove to be the case, but here the question arises as to whether the common antigenic substance or substances were (1) unmodified plant protein present in the crude extract and not entirely removed by the different methods of purification employed; (2) antigenically altered plant protein; or (3) the virus, *per se*, present in both crude and purified preparations.

Experiments were conducted in which antisera were prepared for virus material purified by the three methods already described. The precipitin tests, utilizing crude juice of healthy plants as an antigen in addition to the virus preparations, indicated that the purified virus preparations contained a common antigenic factor which was not present in the juice of healthy plants. This fact is of particular significance from two points of view. First, the methods of virus purification employed eliminate all or practically all unmodified plant proteins which may serve as antigens; second, the antigenic substance which gives rise to positive reactions with these antisera is not present in healthy plants.

The possibility that the common factor present in crude and purified preparations of virus is antigenically altered plant protein must be considered. However, inasmuch as virus purified by acetone precipitation and by tryptic digestion from tobacco as well as virus purified by $Al(OH)_3$ adsorption from tomato were used as antigens, it seems improbable that the antigenic factor common to the purified preparations was antigenically altered plant protein.

Filtration experiments

During filtration experiments with Tobacco Virus I Johnson, it was noticed that the Seitz E. K. filter[2] removed virus, and that the filtrate was not infectious until several hundred cubic centimeters of juice had passed the filter. Since the virus was held back by the filter while most of the plant protein had passed through, it would seem that if the precipitin reaction is due to altered plant protein, the Seitz filtrate should give a precipitin test, unless the virus alters the protein so that it is not filterable. However, if the positive precipitin test is due to the virus, no reaction would be expected.

An experiment was conducted to test this hypothesis. Juice from mosaic tobacco plants was cleared by filtration through a Berkefeld V filter and divided into three portions, one of which was used without further treatment as crude virus I antigen. The second portion was filtered through a Seitz filter and used as antigen crude Seitz filtrate. The third was treated with $Al(OH)_3$, according to the method of Brewer et al., to remove the plant proteins, and again divided

[2] The Seitz filters used were of 100 cc. capacity. Two pads were used.

into two portions. One part was filtered through a Berkefeld V filter and used as antigen $Al(OH)_3$ Berkefeld filtrate; this filtrate was infectious. The other was passed through a Seitz filter and used as antigen $Al(OH)_3$ Seitz filtrate; it was not infectious.

The antiserum used was obtained from a rabbit immunized with a purified preparation of tobacco mosaic virus from tomato.

The results given in table III tend to confirm the hypothesis that the precipitin reaction depends upon the presence of the virus, since

TABLE III

EFFECT OF SEITZ FILTRATION ON PRECIPITIN REACTION OF SELECTED ANTIGENS

o = NO REACTION, + = DEFINITE PRECIPITATE

ANTISERUM (DILUTION 1:4)	ANTIGENS (DILUTION 1:2)				
	CRUDE MOSAIC	SEITZ FILTRATE OF CRUDE MOSAIC	TOBACCO MOSAIC $AL(OH)_3$ BERKEFELD FILTRATE	TOBACCO MOSAIC $AL(OH)_3$ SEITZ FILTRATE	HEALTHY PLANT
Crude mosaic virus I....	+	+	+	o	+
$Al(OH)_3$ tobacco mosaic..	+	o	+	o	o
Healthy plant.........	+	+	o	o	+

the purified virus antiserum reacts with crude or purified antigens which contain the virus but not with the Seitz filtrates of purified virus which do not contain the virus. Seitz filtrates of crude virus juice react with the healthy plant antiserum, indicating that the plant protein passes the filter.

RESULTS OF PRECIPITIN TESTS

1. Crude extracts of healthy and mosaic-diseased tobacco plants have antigenic substances in common.

2. Crude extracts of mosaic-diseased plants and purified preparations of the crude extracts have antigenic substances in common.

3. Antisera prepared for the virus purified by three different methods show a common factor which appears to consist of the virus, or a complex containing the virus, since the antisera reacted with

crude virus-containing plant juice but not with the crude juice of healthy plants.

4. Purified preparations of tobacco mosaic virus from tomato plants reacted with antisera for crude virus-containing juice from tobacco plants and with the antisera for purified preparations, but not with juice from healthy tobacco plants, indicating that the reactions secured were due to the virus and not to the plant protein.

5. Berkefeld filtrates of purified virus were infectious and reacted with the antisera prepared against virus-containing antigens.

6. Seitz filtrates of crude virus were not infectious and did not react with the antisera prepared for the purified virus, but did react with the crude mosaic antiserum and with healthy plant antiserum.

The results of PURDY and of MATSUMATO and SOMAWAZA on the virus of tobacco mosaic are thus confirmed. In addition, evidence has been obtained that the precipitin reactions secured are specific for the virus, or a complex containing the virus, since similar results are obtained regardless of the method employed in purification of the virus.

Part II

PROBLEM

An attempt was made to differentiate several plant viruses by the precipitin test. The viruses[3] used were: spot necrosis (Tobacco Virus IV Johnson), ring spot (Tobacco Virus V Johnson), tobacco mosaic (Tobacco Virus I Johnson), and attenuated forms of spot necrosis and tobacco mosaic (5).

EXPERIMENTAL PROCEDURE

It was soon discovered that the various methods used for purification of tobacco mosaic are not adequate for purification of other viruses, so that considerable experimentation on methods of purification was necessary. In the preparation of antigens of spot necrosis, ring spot, and attenuated spot necrosis, precipitation of the virus with safranin and removal of the safranin by adsorption with Lloyd's reagent according to the method described by VINSON (11) proved most satisfactory.

[3] Professor JAMES JOHNSON of the Department of Horticulture, University of Wisconsin, kindly supplied the pure strains of the viruses used in this study.

PURIFICATION BY SEITZ FILTER METHOD.—The antigen of Tobacco Virus I was purified and concentrated by adsorption on a Seitz filter and subsequent elution with buffers. Since this method has not been reported previously it is described here in detail. Adsorption of the virus of tobacco mosaic by the Seitz filter, already noted, had suggested the possibility of using it to collect, purify, and concentrate the virus. Accordingly diseased plants were ground in a food chopper, the crushed material frozen and thawed, and the juice pressed out and centrifuged until clear. The supernatant fluid (the crude plant juice) was first passed through a Berkefeld V filter to remove materials not precipitated by centrifuging, and then passed through a Seitz filter. To remove the accumulated plant material from the pad, the Seitz filter was washed with about 200 cc. of distilled water, or until the filtrate was clear and colorless. Experiments on the infectivity of the filtrate show that distilled water does not remove the adsorbed virus from the pad to any appreciable extent.

Filtration is a complex phenomenon and many factors other than pore size are involved. Perhaps the two most important factors determining the filterability of particles are (1) the charge of the particle and of the filter, and (2) the surface tension and the pH of the menstrum. In order to determine which of these factors caused Tobacco Virus I to be adsorbed on the Seitz filter pad, three methods of releasing it were employed: electrophoresis, use of surface tension depressants, and change of pH with buffers.

If the viruses are adsorbed on the Seitz filter because of a difference in the charge of the virus (1) and the filter, it would seem possible to suspend the adsorbed virus in an electrolyte and to release it by electrophoresis. Consequently Seitz filter pads through which 500 cc. of mosaic juice had been passed were ground in 50 cc. of 0.85 per cent NaCl solution. This suspension was placed in a U-tube and electrodes carrying 110 volts of direct current introduced. Samples of the suspension were removed from both poles at 2-, 4-, and 20-hour intervals. The samples from the negative pole contained no virus and those from the positive pole only a small amount, as evidenced by their infectivity when tested by HOLMES's method. The experiment was repeated on the other viruses with

similar results. It is possible that the virus may have been inactivated or killed by the concentration of chlorine liberated during electrolysis.

Since electrophoresis did not effectively separate the virus from the filter, ethyl alcohol, as a surface tension depressant, was next tried. After filtering 500 cc. of Tobacco Virus I, the Seitz filter was washed alternately with 40 cc. of 95 per cent ethyl alcohol and 10 cc. of water until a total of 100 cc. of liquid had been passed through it. The alcohol was removed from the filtrate by distillation *in vacuo* at 40° C., and the remaining suspension diluted to 500 cc. and tested for infectivity on *Nicotiana glutinosa*. The three plants inoculated with crude untreated juice showed an average of eighteen lesions per leaf, while the three plants inoculated with the alcohol-free filtrate showed an average of forty-five lesions per leaf, thus indicating a relatively high concentration of virus.[4] The experiment was repeated using methyl alcohol with essentially the same results. The method seems to offer possibilities for the purification and concentration of Tobacco Virus I, which is very resistant to alcohol. However, when the experiment was repeated with spot necrosis, attenuated spot necrosis, and ring spot, the viruses were evidently inactivated or killed by the alcohol.

Finally, elution of the viruses was attempted by the use of SÖRENSEN'S phosphate buffers at pH 6.0, 6.3, 6.6, 7.0, 7.2, 7.6, and 8.2. It seemed probable that since adsorption depends to some extent upon pH, the virus might be released from the filter by the use of buffers. Two hundred cc. of crude mosaic juice was passed through a Seitz filter. The filter was washed with distilled water until the filtrate was clear and colorless, the pads were removed and macerated in 70 cc. of the buffer, and placed in the icebox overnight. This last step was not essential. The pulp was then separated from the virus suspension by filtration through a Gooch asbestos filter. While the results were not always consistent, buffers with a pH of 6.6–7.2 seemed most effective in eluting the virus. Suspensions

[4] The apparent increase of the virus obtained might be explained on the assumption that the infectious agent exists as aggregates, or is adsorbed on particles that exist as aggregates which are broken up during manipulation.

thus obtained were colorless but slightly opalescent. That they contain a high concentration of virus, as tested by HOLMES's *Nicotiana glutinosa* method, is indicated by the following protocol.

INOCULUM	AVERAGE NUMBER OF LESIONS ON N. GLUTINOSA
Crude virus	16
Virus purified by the Seitz filter method at pH 7 and diluted to 200 cc.	69
Seitz filtrate	4

It will be noted that the Seitz filtrate was slightly infectious. After filtering large amounts of virus, the Seitz filter becomes saturated and some virus passes it, although there seems to be great

TABLE IV

PRECIPITIN TESTS WITH TOBACCO VIRUS I ANTISERA AND PURIFIED HOMOLOGOUS AND HETEROLOGOUS ANTIGENS

?=INDECISIVE, o=NO REACTION, +=FAINT PRECIPITATE, ++=SLIGHT BUT DEFINITE PRECIPITATE, +++=MODERATE PRECIPITATE, ++++=HEAVY PRECIPITATE

(READINGS WERE MADE AFTER 8 AND 24 HOURS. INCUBATION, 8 HOURS AT 37° C., 16 HOURS AT ICEBOX TEMPERATURE)

PURIFIED ANTIGENS	ANTISERUM TOBACCO VIRUS I PURIFIED BY SEITZ FILTER METHOD							CONTROLS	
	DILUTIONS							NACL	NORMAL SERUM
	1:2	1:4	1:8	1:16	1:32	1:64	1:128		
Tobacco Virus I Seitz filter method	++++	++++	++	++	o	o	o	o	o
Spot necrosis Vinson's method	o	o	o	o	o	o	o	o	o
Attenuated spot necrosis Vinson's method	?	o	o	o	o	o	o	o	o
Healthy plant Vinson's method	o	o	o	o	o	o	o	o	o
Ring spot Vinson's method	o	o	o	o	o	o	o	o	o

variation in the ease with which this is accomplished. In general the juice from old plants or from plants showing severe symptoms is more effective in saturating the filter than is the juice from young

actively growing plants. Furthermore, if the filter is saturated with paraffin oil, the virus will readily pass it.

This method of purification by adsorption of the virus on the Seitz filter and subsequent elution by the use of buffers, hereafter

TABLE V

PRECIPITIN TESTS WITH SPOT NECROSIS ANTISERUM AND PURIFIED HOMOLOGOUS AND HETEROLOGOUS ANTIGENS

o = NO REACTION, + = FAINT PRECIPITATE, ++ = SLIGHT BUT DEFINITE PRECIPITATE, +++ = MODERATE PRECIPITATE, ++++ = HEAVY PRECIPITATE

PURIFIED ANTIGENS	ANTISERUM SPOT NECROSIS							CONTROLS	
	DILUTIONS							NACL	NORMAL SERUM
	1:2	1:4	1:8	1:16	1:32	1:64	1:128		
Spot necrosis Vinson's method...	++++	++++	++++	++++	++++	+++	+++	+	o
Attenuated spot necrosis Vinson's method...	++	++	++	+	+	+	+	o	o
Healthy plant.....	o	o	o	o	o	o	o	o	o
Tobacco Virus I Seitz filter method...	o	o	o	o	o	o	o	o	o
Ring spot Vinson's method...	++	++	++	++	o	o	o	o	o

designated as the Seitz filter method, was used in preparing Tobacco Virus I; and in a later experiment, in purifying cucumber mosaic. It has several advantages: it is simple and rapid, and does not involve the use of chemicals harmful to the virus.

PRODUCTION OF ANTISERA.—The antigens of Tobacco Virus I, purified according to the Seitz method; of spot necrosis, attenuated spot necrosis, and ring spot, purified according to VINSON'S method; and of a healthy plant were given to rabbits in a series of eleven injections of 3 cc. each. On the sixth day after the last injection the rabbits were exsanguinated, the blood allowed to clot, and the sera collected.

PRECIPITIN TESTS.—Precipitin ring tests were set up with homologous and heterologous antigens. The results were not satisfactory as the titres were too low. This was attributed to the fact that the antigens were not concentrated enough to allow dilution.

Regular precipitin tests were then run. In this case the dilutions of antigen were held constant and those of the antisera varied. The

TABLE VI

PRECIPITIN TESTS WITH ATTENUATED SPOT NECROSIS ANTISERUM AND PURIFIED HOMOLOGOUS AND HETEROLOGOUS ANTIGENS

o = NO REACTION, + = FAINT PRECIPITATE, ++ = SMALL BUT DEFINITE PRECIPITATE, +++ = MODERATE PRECIPITATE, ++++ = HEAVY PRECIPITATE

Purified antigens	Antiserum Attenuated spot necrosis							Controls	
	Dilutions							NaCl	Normal serum
	1:2	1:4	1:8	1:16	1:32	1:64	1:128		
Attenuated spot necrosis Vinson's method....	++++	++++	++++	++++	+++	++	++	o	o
Spot necrosis Vinson's method....	++++	++++	++++	+++	++	+	+	o	o
Ring spot Vinson's method.........	++	++	++	++	o	o	o	o	o
Tobacco Virus I Seitz filter method....	o	o	o	o	o	o	o	o	o
Healthy plant	o	o	o	o	o	o	o	o	o

sera were diluted with 0.85 per cent NaCl. Each precipitin tube contained 0.5 cc. of serum and 0.5 cc. of antigen.

Table IV shows that antisera for Tobacco Virus I, purified according to the Seitz method, reacts with its homologous antigen but not with antigens of the other viruses nor with the antigen of a healthy plant. This indicates that there is no serological relationship between Tobacco Virus I and the other viruses, and that the normal plant antigen had been removed by the purification methods employed.

Tables V, VI, and VII show (1) that the purified preparations of

spot necrosis, attenuated spot necrosis, and ring spot are antigenic; (2) that these viruses are not related serologically to Tobacco Virus I; and (3) that there appears to be a serological relationship between the viruses of ring spot and of spot necrosis. No significant serological difference is evidenced between spot necrosis and attenuated spot necrosis.

TABLE VII

PRECIPITIN TESTS WITH HEALTHY TOBACCO ANTISERUM AND HOMOLOGOUS AND PURIFIED HETEROLOGOUS ANTIGENS

Purified antigens	Antiserum Healthy tobacco plant							Controls	
	Dilutions							NaCl	Normal serum
	1:2	1:4	1:8	1:16	1:32	1:64	1:128		
Healthy plant.	++++	++++	++++	++++	++	++	?	0	0
Tobacco Virus I Seitz filter method.....	0	0	0	0	0	0	0	0	0
Spot necrosis Vinson's method.....	++	++	0	0	0	0	0	0	0
Attenuated spot necrosis Vinson's method.....	++	++	0	0	0	0	0	0	0
Ring spot Vinson's method	0	0	0	0	0	0	0	0	0

PRECIPITIN ABSORPTION TESTS

In order to check further the results of precipitin tests with purified antigens, precipitin absorption tests were run on the antisera prepared against the following antigens:

1. Tobacco mosaic Virus I, purified by the Seitz filter method.
2. Tobacco mosaic Virus I, crude.
3. Attenuated tobacco mosaic, Virus I, attenuated by growing plants at a high temperature.
4. Spot necrosis.
5. Ring spot.[5]
6. Cucumber mosaic, purified by the Seitz method.

[5] This strain was obtained from Dr. R. G. HENDERSON of the Department of Plant Pathology, Virginia Agricultural Experiment Station, Blacksburg.

7. Crude cucumber juice, cleared by supercentrifuging at 33,000 r.p.m.
8. Healthy tobacco plant, cleared by passing through Berkefeld V filters.

TABLE VIII

SUMMARY OF PRECIPITIN TESTS WITH ABSORBED SERA IN THE PRESENCE OF HOMOLOGOUS AND HETEROLOGOUS ANTIGENS

o = NO PRECIPITATE, + = SLIGHT PRECIPITATE, ++ = DEFINITE PRECIPITATE, +++ = MODERATE PRECIPITATE, ++++ = HEAVY PRECIPITATE

Antigens (dilution 1:3)	Tubes	Antisera (dilution 1:10)							
		Tobacco Virus I, Seitz filter method	Virus I, crude	Attenuated mosaic	Spot necrosis	Ring spot	Cucumber mosaic, filter method	Cucumber mosaic, crude	Healthy tobacco plant
Tobacco Virus I, Seitz filter method	1	++++	++++	+++	o	o	o	o	o
	2	++++	++++	+++	o	o	o	o	o
Tobacco Virus I, crude	1	++	++	++	o	o	o	o	o
	2	+++	++	++	o	o	o	o	o
Attenuated mosaic	1	+++	++	+++	o	o	o	o	o
	2	++	++	+++	o	o	o	o	o
Spot necrosis	1	o	o	o	+	o	o	o	o
	2	o	o	o	+	o	o	o	o
Ring spot	1	o	o	o	o	o	o	o	o
	2	o	o	o	o	o	o	o	o
Cucumber mosaic, Seitz filter method	1	o	o	o	o	o	+	++	o
	2	o	o	o	o	o	+	++	o
Cucumber mosaic, crude	1	o	o	o	o	o	+	+	o
	2	o	o	o	o	o	+	+	o
Healthy tobacco plant	1	o	o	o	o	o	o	o	+++
	2	o	o	o	o	o	o	o	+++

Rabbits were immunized as previously described to each of these antigens. The antisera thus produced were absorbed with healthy plant antigen as follows. One and five-tenths cc. of a healthy plant was added to 3 cc. of each antiserum, the mixture incubated at 37° C. for four hours, placed in an icebox overnight, and centrifuged. The

supernatant liquid was pipetted into sterile test tubes and 1.5 cc. of healthy plant antigen was again added. This process of absorption was repeated until no precipitate formed upon the further addition of healthy plant antigen.

Precipitin tests

The absorbed antisera were then tested for precipitins with homologous and heterologous antigens. Five-tenths of a cubic centimeter of 1:10 dilution of antiserum was added to 0.5 cc. of 1:3 dilution of antigen. These dilutions of antigen and antiserum were employed because preliminary experiments had shown that if cross-reactions occurred, they were most likely to appear under these conditions.

The results of these tests, summarized in table VIII, indicate that when precipitins of a healthy plant have been removed from the antiserum by absorption with healthy plant antigen, the serum gives a positive test only with the homologous virus antigen. This confirms the results of tests with purified antigens. No difference could be detected between Tobacco Virus I and an attenuated form. The absorbed ring spot antiserum did not give any reaction with homologous or heterologous antigen. The titre of the ring spot antisera with homologous antigen was 1:8, consequently the dilution that resulted when the serum was absorbed was too great to give a reaction.

Summary

1. The juice from virus-diseased plants contains, in addition to the antigenic constituents of a normal healthy plant, an antigenic fraction which, by the methods employed, is inseparable from the virus itself. This holds true in the case of Tobacco Virus I whether the virus is grown in tobacco or in tomato. This antigenic factor not only accompanies the virus but is specific for a particular virus, in that the antibodies induced by one virus are qualitatively different from those induced by other viruses.

2. Viruses may be freed from the antigenic constituents of healthy plants by several methods of purification. A new method by use of a Seitz filter is described. Regardless of the method of purification, however, it was not possible to separate the virus, as judged by infectivity, from the specific antigenic factor which accompanies it.

3. Close association of the antigenic factor with infectivity and

the specific nature of the antigenic fractions accompanying the different viruses strongly suggest that this specific antigenic factor is either the virus itself or a virus-plant-protein complex in which the virus plays the rôle of a haptene. Whatever the true explanation of the nature of the specificity of the reaction may be, the reaction seems to be specific for the virus. The precipitin test should prove to be a valuable aid in the further classification of plant viruses.

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AMMONIUM AND NITRATE NUTRITION OF DORMANT DELICIOUS APPLE TREES AT 48° F.[1]

G. T. NIGHTINGALE

Introduction

In previous publications (6, 7, 8, 9) it has been repeatedly pointed out that plants may absorb large quantities of nitrate nitrogen; but unless nitrates first combine with carbohydrate materials to form proteinaceous compounds, there is little apparent effect upon the growth of the plant. Recent work by TIEDJENS (18, 19, 20) has also indicated that absorption of ammonium nitrogen by plants does not result in the formation of new cells and growth unless followed by the assimilation or synthesis of the ammonium nutrient to organic compounds of nitrogen.

In the process of protein synthesis from nitrates, there are found in the plant in successive stages nitrites and ammonium nitrogen (1). Obviously, on a theoretical basis, ammonium compounds should therefore be more rapidly assimilated by the plant than nitrate containing nutrients. TIEDJENS (18, 20) has demonstrated that, for Delicious apple trees in sand culture under usual greenhouse temperature conditions, the most favorable initial pH of the nutrient solution, for assimilation of the nitrate ion was about 4.5, and for the ammonium ion about 6.0.

THOMAS (16) and ECKERSON (2) have demonstrated that in apple trees the fine fibrous roots are the organs chiefly responsible for the initial stages in protein synthesis. It is a fairly common practice in some fruit growing sections to apply nitrogenous fertilizers to the orchard in the early spring, before the leaves have expanded and while soil and air temperatures are still comparatively low. With this in mind, the present work was undertaken to determine the relative rate of availability of ammonium and nitrate nitrogen in the case of

[1] Through the courtesy of the University of Chicago there was made available for this work the temperature control equipment and laboratory facilities of its Department of Botany. The writer wishes also to express his appreciation of the advice and cooperation of E. J. KRAUS and M. A. BLAKE.

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Delicious apple trees at a temperature of 48° F. This temperature, as will be shown in a later publication, probably approaches, for many varieties of apples, the lower limit of possible assimilation of nitrogenous nutrients.

Experimental methods

During the summer of 1932, about 300 specially selected 18-inch, 1-year-old Delicious apple trees, which had been root grafted on seedling roots, were grown in the greenhouse in sand culture. The trees were cut back to a uniform height of 12 inches when placed in the sand, and all buds except one were removed. For the first few weeks the trees received a complete nutrient solution, until the single shoot upon each tree had attained a growth of about 12 inches. At that time the nutrient treatment was changed, so that the trees received no external nitrogen supply but did receive all other nutrients. At the end of the summer the trees exhibited the usual symptoms of nitrogen deficiency. The foliage was light green with reddish petioles, and the bark was yellowish in color except near the terminal growing point where it was reddish yellow. The current twig growth was relatively short, of small diameter, stiff and lignified, and practically all parenchymatous tissue throughout the trees was packed with starch grains. In proportion to tops the trees had an enormous volume of very fine white fibrous roots that appeared nearly to fill the 12-liter self-draining porcelain jars in which they were grown, three trees per jar.

At the end of the summer, after all the leaves had abscissed, the crocks and trees were shifted intact to a cold storage cellar at 35°–40° F., where they remained until January 6, 1933, with no treatment other than application of tap water as required to keep the sand constantly moist. On that date the trees were removed from the crocks and the current stem cut back to two buds. All the fine fibrous roots were removed, so that there remained only old roots, none of which was less than 1 cm. in smallest diameter.

The trees were then selected for uniformity and reset in nitrogen-free white quartz sand in new 10-inch clay pots, three plants per pot. They were grown in a greenhouse at about 50° F. and received daily applications of minus-nitrogen nutrient solution (table I) in suffi-

cient amount to flush the sand of each culture. On February 12, 56 trees (hereafter referred to as initial trees) were harvested for analysis. The two buds that remained after the pruning treatment already described had expanded to the extent that leaves were visible and were about 1 cm. in length. During the same period roots had developed rapidly. The first formed new roots were from 8 to 16 cm. in length and had many laterals. The entire new fibrous root system was white and succulent with a green weight of 4.8 gm. per tree.

TABLE I

COMPOSITION OF NUTRIENT SOLUTIONS. PARTIAL VOLUME MOLECULAR CONCENTRATIONS OF SALTS USED

Series	pH	$Ca(NO_3)_2$	$(NH_4)_2SO_4$	$CaCl_2$	KH_2PO_4	$MgSO_4$
Minus N..........	5.0	....	....	0.0090	0.0045	0.0045
Ammonium N......	6.0	....	0.0045	0.0045	0.0045	0.0045
Nitrate N.........	4.5	0.0090	....	....	0.0045	0.0045

At the time of initial analysis the remainder of the trees were placed in continuous darkness in a cold storage room at 48° F. (±0.5). They were divided into three groups each containing 30 trees. Each group received the respective nutrient solution indicated in table I. The solutions were percolated through the sand according to the constant renewal method of SHIVE and STAHL (15) at the rate of 36 liters per culture every 24 hours. This rate of renewal was such that the solution, after passing through the sand, did not vary more than 0.1(±)pH from that initially supplied (table I). The same solution was passed through the sand three times daily, the necessary adjustments of the pH being made after each passage with 0.1 N KOH or H_2SO_4 as required. At the end of 24 hours the solution was replaced by nutrient not previously used. This treatment was continued for 16 days, at 48° F.

In addition, six of the trees of the ammonium sulphate group and a similar number of the calcium nitrate group were grown in water cultures instead of in sand. The solutions of this lot were also changed at the rate of 36 liters per culture per day, and in addition each water culture was vigorously stirred with a motor-driven agitator so that the new white fibrous roots were in constant gentle waving motion.

CHEMICAL METHODS

PLANT FRACTIONS.—The new fine fibrous roots, the analyses of which are given in table II, consisted of new roots only. They were white, very succulent, relatively young, and not more than 0.2 cm. in diameter (most of them were less). Because of the pruning treatment already described, no roots were present which were intermediate between new fibrous roots and old large roots. The old large roots, together with the wood growth of 1931 and that of 1932, are included as a single analytical sample (table II). The two buds per tree, which were slightly expanded, were analyzed as a third analytical sample; and the results of the analyses of these buds, in so far as they are pertinent, are given in the context.

NITROGENOUS FRACTIONS.—The new fine fibrous roots were minced with a large knife and thoroughly mixed. Aliquots were boiled for 20 minutes (21), following which they were ground with a mortar and pestle and extracted. Nitrogenous fractions were determined as previously described (10, 11), with the single exception that ammonium nitrogen was determined by aeration of the coagulum-free extract with 6 per cent NaOH. The old large roots and tops were rapidly cut in a specially constructed mill and the resultant thin shavings were boiled and extracted in the same manner as the roots.

The term organic nitrogen is synonymous with ammonium and nitrate-free nitrogen.

The pH estimations were made colorimetrically, using as standards the colored glass discs of a Hellige Klett color comparator.

Discussion of results

During the 16-day period at 48° F. the buds expanded slightly, but made little if any measurable growth. The roots grew rapidly, however, and for the period of the experiments were essentially the same in appearance, weight, and volume under all nutrient treatments. The average green weight of new, fine fibrous roots was about 9 to 10 gm. per tree when finally harvested for analysis, as contrasted with 4.8 gm. at the time of commencing experimental treatment 16 days previously. In the water cultures the growth of individual roots was easily observed and measured, and it was found that some elongated 1–2 cm. in 24 hours. This is in striking contrast to the

TABLE II

DORMANT DELICIOUS APPLE TREES GROWN IN SAND CULTURE AT 48° F. AND CONSTANT PH VALUES (NITROGENOUS FRACTIONS AND DRY MATTER EXPRESSED AS PERCENTAGE OF GREEN MATTER)

	NEW FINE FIBROUS ROOTS							OLD LARGE ROOTS AND TOPS						
	INITIAL	8 DAYS			16 DAYS			INITIAL	8 DAYS			16 DAYS		
	pH 5.0 MINUS N	pH 5.0 MINUS N	pH 4.5 $Ca(NO_3)_2$	pH 6.0 $(NH_4)_2SO_4$	pH 5.0 MINUS N	pH 4.5 $Ca(NO_3)_2$	pH 6.0 $(NH_4)_2SO_4$	pH 5.0 MINUS N	pH 5.0 MINUS N	pH 4.5 $Ca(NO_3)_2$	pH 6.0 $(NH_4)_2SO_4$	pH 5.0 MINUS N	pH 4.5 $Ca(NO_3)_2$	pH 6.0 $(NH_4)_2SO_4$
Dry matter...	7.060	7.850	6.370	6.090	7.910	6.060	4.950	26.500	26.750	25.500	25.750	25.800	24.720	24.000
Total organic N	0.102	0.109	0.274	0.333	0.110	0.380	0.616	0.081	0.077	0.082	0.087	0.084	0.089	0.094
Protein N.....	0.073	0.076	0.164	0.149	0.092	0.183	0.216	0.048	0.047	0.045	0.049	0.051	0.051	0.054
Soluble organic N..........	0.029	0.033	0.110	0.184	0.018	0.197	0.400	0.033	0.030	0.037	0.038	0.033	0.038	0.040
Amino N.....	0.004	0.004	0.045	0.077	0.003	0.067	0.120	0.002	0.002	0.003	0.003	0.002	0.004	0.005
Amide N.....	0.003	0.004	0.021	0.040	0.002	0.049	0.076	0.002	0.002	0.002	0.003	0.001	0.003	0.003
Ammonium N.	None	None	0.002	0.006	None	0.003	0.008	None	None	Trace	Trace	None	Trace	Trace
Nitrate N.....	None	None	0.011	None	None	0.014	None	None	None	None	None	None	None	None

extremely slow growth of the aerial organs, which grew rapidly, however, when a few of the trees were shifted to a temperature of 70° F.

The pH values selected for the ammonium and nitrate cultures (table I) were not chosen arbitrarily, but are those found by TIEDJENS (18, 20) to be favorable for the growth of Delicious apple trees in sand culture when the nutrient solution was maintained at a practically constant pH value. As usual (5, 12, 13, 19), the ammonium sulphate cultures tended to become more acid, a change which is generally considered (13) to be due mainly to the more rapid absorption of the ammonium than of the sulphate ion; and the calcium nitrate nutrient solutions became more alkaline owing to less absorption of the calcium than of the nitrate ion. As already pointed out, the solution of each culture constantly underwent renewal at the rate of 3 liters per hour; and as a result the solution, after passing through the sand, did not vary more than 0.1 pH from that initially supplied. Nevertheless the trees receiving the ammonium sulphate at pH 6.0 had, at the absorbing surface and tips of the fine fibrous roots, a pH of 4.0 to 4.5. The sand immediately adjacent to the roots also had about the same H-ion concentration, with a gradient reaching pH 6.0 at about 2 cm. from the absorbing surface of the root.

The calcium nitrate cultures at pH 4.5 had, at the absorbing surface and tips of the fine roots, a pH of 5.6, as did also the sand in contact with the roots; although less than 1 cm. from the roots the solution on the sand particles was about pH 4.5. On the other hand, the minus-nitrogen cultures at pH 5.0 did not noticeably affect the H-ion concentration of the solution as a whole, and the surface of the fine roots appeared to have a pH value of approximately 4.8–5.0. Yet the roots, although somewhat more slender, were growing (at least in length) at about the same rate as in the case of the trees receiving nitrogen in the nutrient solution.

Both the ammonium and the nitrate series were duplicated in water culture. The solutions which bathed the roots underwent constant renewal at the rate of 3 liters per hour and were continuously and vigorously stirred with a motor-driven agitator. Even under these conditions the ammonium sulphate (pH 6.0) cultures had at the surface of the fine absorbing roots a capillary film of pH 5.4,

while the trees supplied with calcium nitrate nutrient solution at pH 4.5 had at the surface of the absorbing roots a film of pH 5.2.

It is obviously impossible, in sand culture, absolutely to control the pH of the solution bathing the roots, if the plant is absorbing from the nitrogen-containing salt of the nutrient solution in largest part ammonium from ammonium sulphate or nitrate from calcium nitrate. Under the conditions of water culture, however, in which more extreme changes in pH at the root surfaces were eliminated, the trees were able to grow without injury and produce roots apparently healthy in every way, in a solution containing ammonium sulphate at a partial volume molecular concentration of 0.0180. This is four times as concentrated as that employed in most of the sand culture series (table I). A few of the trees in sand culture, which were subjected to this amount of ammonium sulphate at pH 6.0($\pm$0.1), produced roots which were short, stubby, and bulbous in appearance. This was due mainly to the development of the primordia of branch roots, most of which never developed sufficiently to emerge through the cortex, probably because of the extremely acid condition of the root surface and of the outer cortical cells which were about pH 2.8–3.0.

The preceding results would not indicate that a widely different pH of the nutrient medium is required for ammonium from that required for nitrate assimilation. Actually, as is shown in table II, the trees of the ammonium series assimilated nitrogen rapidly when the solution bathing the roots was pH 4.5, and those of the nitrate series when the absorbing surface of the roots was pH 5.6. Practically, under conditions of sand culture it is essential that the initial solution containing ammonium sulphate be approximately neutral (pH 6.0), and that that of a calcium nitrate culture be relatively acid (pH 4.5), in order that the absorbing root surfaces may not become extremely acid in the former case or excessively alkaline in the latter case. Actually in practice (17) there has been some indication that ammonium sulphate may be assimilated efficiently by fruit trees growing in a soil of pH 4.2, which was not high in soluble aluminum or other toxic materials. This is not surprising, however, as it is well known that the H-ion concentration under usual conditions changes slowly

in a well buffered soil. Obviously, however, it would be a short sighted policy to apply ammonium sulphate season after season to an acid soil, without liming. The soil would gradually lose in buffer capacity and increase in acidity, owing partially to the greater absorption of the ammonium than of the sulphate ion.

This change in pH of the solution at the surface of the absorbing roots explains certain phenomena observed by others working with sand cultures. While it is not actually a part of this investigation, it may be worth while to point out that TIEDJENS (19, 20) found that the plants of his initially acid calcium nitrate cultures frequently exhibited symptoms of iron deficiency, whereas the plants of his initially alkaline ammonium sulphate series did not. At first thought this would seem illogical as iron is relatively soluble in acid but insoluble in alkaline solution (14). Even though applied at pH 6.0, however, the solution bathing the roots of the ammonium sulphate trees became relatively acid, which would tend to keep iron in solution, as compared with the calcium nitrate cultures which, although supplied with solution at pH 4.5, became alkaline at the root surfaces, thereby tending to precipitate iron out of solution.

Some studies were made of the pH of the internal tissues, especially of the new white fibrous roots. The pH of the tissues apparently did not vary directly with the nutrient solution, except in the case just described where the trees in sand culture were subjected to ammonium sulphate at a partial volume molecular concentration of 0.0180, and in that case the roots were seriously injured. The pH values of comparable tissues of the roots of the ammonium sulphate series at pH 6.0 were essentially the same as those of the trees with no external nitrogen supply at pH 5.0, or those receiving calcium nitrate at a H-ion concentration of 4.5. The epidermis and outer two or three layers of cortical cells of the absorbing roots may have been slightly modified in the direction of the pH of the solution bathing the roots, but if so it was very slight and not true of the inner cortical cells.

The cortex varied in pH from 5.2 to 4.4, or even 4.2 in occasional cells, but the lower two figures were invariably associated with heavy deposition of starch (as in roots of the minus-nitrogen cultures), not with the pH of the nutrient medium. The phloem and cambium

were relatively alkaline, about pH 5.6–5.8, except in the case of the phloem rays which were about pH 4.6 when starch was present. The mature xylem tissue appeared to have a H-ion concentration of about 4.8–5.2, but in the vicinity of the cambium the recently developed xylem elements were approximately 5.6.

Limited examination of the old, large roots and tops also indicated a similar situation; the starch storing tissues were relatively acid and, as in the smaller roots, of a pH value approaching that usually considered optimum for diastatic digestion (pH 4.2). Relatively young tissues and the phloem, except in starch storing cells, were comparatively alkaline. This is not unlike the situation already described by ECKERSON (1) for tomato.

When the roots of the initial plants were examined, no asparagine was detected and all the parenchymatous tissue nearly to the root tips was packed with starch grains. As already emphasized, the apple trees of these experiments had been grown for an entire season with no external nitrogen supply. Such plants are notably high in nitrate reducing material (1) and in ability to synthesize organic nitrogen from nitrogenous nutrients (3). The apple trees of these experiments were apparently no exception, even though they were subjected to a temperature of 48° F.

Nitrate, when applied to these initial plants, was apparently absorbed instantly and remained in the fine roots exclusively. In about two days after application strong reactions for nitrite and ammonium were obtained, accompanying which there was definite etching of starch grains in the smaller roots, and a little later asparagine appeared. Nitrites were absent as usual, or present only in traces, following the first period of rapid nitrate reduction.

In the roots of the ammonium supplied plants no nitrate nor nitrite could be detected at any time, but ammonium was apparently absorbed instantly and was present in quantity only in the new fine roots. During the first two or three days little difference was observed in the starch and asparagine contents of the roots of the two complete nutrient series, but after eight days starch was practically absent from the fine roots of the ammonium supplied plants and did not again appear. Yet there was continuously an abundance of starch in comparable roots of the nitrate cultures, although very

much less than in the plants which received no external nitrogen supply.

Accompanying the almost complete disappearance of starch from the fine roots of the ammonium supplied trees, asparagine appeared in much larger quantity than could be detected in the fine roots of the trees receiving nitrate. No asparagine[2] was observed in the minus-nitrogen treated trees.

The situation observed microchemically is also in close agreement with the results of macroanalysis (table II). At the end of 16 days total organic nitrogen in the new fine roots of the ammonium supplied trees was nearly twice as high in concentration as in the fine roots of the nitrate cultures. That nitrate was also vigorously assimilated at this low temperature, however, is evident from the fact that there was at time of final harvest about three times as much organic nitrogen in the fine roots of the nitrate series as in these same organs of trees which received no external nitrogen supply. It is also notable that of this total organic nitrogen the soluble fractions accumulated in higher concentration than the more complex proteins. This is especially true in the case of the ammonium supplied trees (table II) and appears to be generally (5, 18, 19, 20) true of plants receiving ammonium as compared with nitrate nutrition.

Tiedjens (18, 20) also analyzed the roots of dormant Delicious apple trees which had been grown in a warm greenhouse with ammonium and nitrate nutrition. Although his analytical sample of roots consisted partly of roots which were older than those of this investigation and therefore somewhat lower in concentration of nitrogen, nevertheless the results here reported are in complete harmony with his work. He likewise found that ammonium was assimilated more rapidly than nitrate, and that the starch content was less and the proportion of soluble organic nitrogen relatively high in the roots of his ammonium supplied trees.

In the synthesis of organic nitrogen from either the ammonium or

[2] It is not intended to indicate that asparagine is of greater significance than other relatively simple compounds of organic nitrogen. It may, however, furnish an index as to the rate of assimilation of ammonium and nitrate nitrogen and may be easily and positively identified (4). It should also be recorded that there were precipitated out in alcohol, along with the asparagine crystals, amino acids which were not identified.

the nitrate ion, it is obvious that there must be a decrease in carbohydrates. That such occurred is evident, not only from the microchemical observations already mentioned, but also from the fact that the roots of the trees receiving nitrogen were lower in percentage dry matter (table II) than those of the minus-nitrogen series, and by far the lowest in dry matter in case of the ammonium sulphate supplied trees. The latter, however, accumulated the greatest quantity of organic nitrogen.

The relatively rapid assimilation of organic nitrogen and accompanying decrease in carbohydrates of the ammonium supplied trees seems logical, for, as has been pointed out, the ammonium ion being further reduced than nitrate should theoretically be more quickly available. These experiments with apple trees at 48° F., together with TIEDJEN'S (**18, 20**) results at higher growing temperatures, indicate that this is true. His work further suggests that ammonium nitrogen may be continuously assimilated by plants as long as carbohydrate materials are available, whereas nitrate reduction or reducase activity (**1, 3**) often lessens as organic nitrogen accumulates and carbohydrates decrease.

Furthermore, there seems usually to be required for nitrate nutrition a much higher concentration of nitrogen in the form of nitrate than of ammonium nitrogen, if equal growth is to be obtained. Repeated trials (**12, 18, 19, 20**) appear to indicate that this is true. For this reason the nitrate cultures employed were purposely made higher in nitrogen than those supplied with ammonium sulphate (table I), the grams of atomic nitrogen per liter of ammonium and of nitrate solution being 0.126 and 0.252 respectively. It is not apparent why there should seemingly be required a greater concentration of nitrate nitrogen, although it is possible that a fairly high percentage of nitrate in the plant is required to furnish ammonium through nitrate reduction at a rate comparable with that made available to the plant in a culture supplied directly with ammonium nitrogen.

During the 16-day period there was no significant increase in organic nitrogen in the old large roots and tops (table II), indicating that at 48° F. there was little if any translocation of the newly synthesized organic nitrogen from the fine roots to the old roots and tops. Obviously, however, there was proteolysis (**6, 7, 8**) and subse-

quent translocation of soluble organic nitrogen from the old roots and tops, at least in the trees of the minus-nitrogen cultures; for these trees had available no external source of nitrogen, yet the new roots continually increased in volume of growth with no loss in percentage of nitrogen. This evident gain in absolute amount of organic nitrogen in the new fibrous roots (table II) was due to translocation from the old roots and tops.

There was a noticeable decrease of starch in the large roots of all the series near the point of attachment of new lateral roots. This was clearly evident in the ammonium sulphate series, somewhat less striking in the nitrate supplied group, and in the minus-nitrogen series was evidenced only by the etching of starch grains.

On a percentage basis the changes in dry matter in old roots and tops were small. It should be remembered, however, that this analytical fraction was made up in large part of old lignified tissues and that decrease in starch was observed to take place in only a small part of the sample as a whole, namely, near the new lateral roots. Large changes in percentage dry matter, in the samples consisting of old roots and tops, could therefore scarcely occur during the 16-day period of these investigations. Although the differences in dry matter are of necessity small (table II), they seem to reflect accurately the carbohydrate changes which necessarily occurred with root development and assimilation of nitrate and ammonium nitrogen.

At the end of 12 days at 48° F., when it was found that there was little or no translocation of the newly synthesized organic nitrogen to the old roots and tops, some of the trees of each series were subjected to a temperature of 70° F. while still in darkness. In 24 hours the buds had noticeably expanded and asparagine in extremely high concentration appeared in their rapidly expanding tissues. These trees were allowed to remain at 70° for four days, when they were harvested at the same time as the comparable series kept continuously at 48° F. During the four days at 70° the buds had expanded so that the leaves, including petioles, were 3–4 cm. in length, whereas the total leaf length of the plants retained at 48° was less than 2 cm.

Neither nitrate nor ammonium nitrogen was detected in the buds or shoots at either high or low temperatures. But Kjeldahl determinations indicated that their total organic nitrogen content on a

percentage basis was, for the buds or shoots of the minus-nitrogen cultures at 48° F., 0.61; for the comparable calcium nitrate group, 0.70; and for the ammonium sulphate series, 0.76. At 70° F. in the same order comparable figures were 0.66, 0.82, and 0.91. These data clearly indicate that, although there was little translocation of newly synthesized organic nitrogen from the roots to the tops at low temperature, there was comparatively rapid movement to the newly developing shoots after the plants were shifted to a temperature of 70° F.

Summary

1. Dormant 2-year-old nitrogen deficient Delicious apple trees were subjected to a constant temperature of 48° F. and continuous darkness. They were grown in sand, and the solutions of each culture underwent renewal at the rate of 36 liters every 24 hours. As a result, after passing through the sand, the solution did not vary more than 0.1 pH from that initially supplied. Trees were also grown with the same nutrient treatments but in water instead of in sand. The water cultures were not only renewed but were constantly stirred with a mechanical agitator.

2. The sole source of nitrogen for some of the trees was a complete nutrient solution containing ammonium sulphate supplied at pH 6.0; for others, calcium nitrate in complete nutrient solution at pH 4.5. Some cultures were also grown with no external nitrogen supply but with other essential elements at pH 5.0.

3. During the 16-day period of these experiments while subjected to a constant temperature of 48° F., buds expanded only slightly but there was vigorous and rapid development of roots in both the sand and the water cultures.

4. Simple proteins were synthesized in the fine fibrous roots of the nitrogen supplied trees. They accumulated apparently exclusively in these organs and were present in much higher concentrations than in the fine fibrous roots of the series grown with no nitrogen in the nutrient solution.

5. The trees which received ammonium sulphate, however, synthesized amino acids and asparagine more rapidly and in greater quantity than did the trees of the calcium nitrate cultures.

6. In the fine fibrous roots of the trees with no external nitrogen

supply carbohydrates increased, undoubtedly owing to translocation. But in those cases where ammonium and nitrate nitrogen were assimilated, there necessarily occurred considerable decrease in carbohydrates, especially in the case of the ammonium supplied trees which were synthesizing organic nitrogen more rapidly than the trees of the nitrate series.

7. On shifting to 70° F., a large part of the newly synthesized amino acids and asparagine was translocated from the fine roots to the buds, which expanded rapidly at the higher temperature.

8. Although the nutrient solutions after passing through the sand did not vary more than 0.1 pH from that initially supplied, the absorbing surfaces of the fine roots of the sand cultures supplied with ammonium sulphate at pH 6.0 were relatively acid (pH 4.2). Those of the calcium nitrate series at pH 4.5 were relatively less acid (pH 5.6). This was presumably due in each case to comparatively rapid absorption of the nitrogen containing ion, with consequent increase or decrease in acidity due respectively to the residual sulphate and calcium ions.

9. From these results it appears that there is not directly required a widely different pH value of the nutrient medium for ammonium as compared with that for nitrate assimilation, provided the absorbing surfaces of the roots are not subjected to extremely acid or alkaline conditions. Practically, however, it would be inadvisable continuously to apply ammonium sulphate to an acid soil, or sodium or calcium nitrate to a very alkaline soil.

10. In a few preliminary experiments in sand culture where higher concentrations of ammonium sulphate were used, there resulted an extremely acid condition of the root surface and outer cortical cells (pH 2.8–3.0). Associated with the acid condition of the cortex many of the root primordia failed to emerge, which resulted in roots that were stubby and bulbous in appearance.

11. In water cultures where the solutions were constantly stirred, similar pH changes occurred but they were much less extreme. Under these conditions where extreme changes in the pH at the root surfaces were eliminated, the roots grew vigorously and assimilated ammonium rapidly, even when present in concentrations four times as high as that in the regular nutrient solutions.

12. Except in the case just described in which injury occurred, the pH of the internal tissues did not vary with that of the nutrient solution. The pH values of respective tissues were essentially the same for all nutrient treatments.

13. The contents of the cells of the cortex of the fine fibrous roots varied in pH from 5.2 to 4.4, or even 4.2 in occasional cells, but the two lower figures were invariably associated with heavy deposition of starch (as in the roots of minus-nitrogen cultures), not with the pH of the nutrient medium.

14. The phloem and cambium were relatively less acid (pH 5.6–5.8) except in case of the phloem rays which were about pH 4.6 when starch was present. Mature xylem appeared to have a H-ion concentration of about 4.8–5.2.

15. Limited examination of old roots and tops also indicated that starch storing cells were relatively acid and meristematic tissues, as the cambium, comparatively alkaline.

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PENNSYLVANIAN FLORA OF ILLINOIS AS REVEALED IN COAL BALLS. I

CONTRIBUTIONS FROM THE HULL BOTANICAL LABORATORY 451

ROY GRAHAM

(WITH PLATES VIII, IX AND TWENTY-SIX FIGURES)

Introduction

This paper is the result of studies made of fossil plants preserved in coal balls from Calhoun coal mine, Richland County, Illinois. The material was collected by Dr. A. C. NOÉ under the auspices of the Illinois State Geological Survey. The mine has now been abandoned, so that no more material can be collected there. The writer is much indebted to Dr. NOÉ, who kindly supplied the coal balls, and under whose direction the research was carried out.

The geologic horizon from which these petrifactions were obtained is about Middle Conemaugh in age, and is near the top of the Pennsylvanian strata found in Illinois.

Thin sections of the material were prepared by employing almost exclusively the cellulose peel process (3). By the use of this method, ten sections of material could easily be obtained to the millimeter, such close sections being almost essential in the study of the small strobili and seeds in order to get sections through critical structures. The numbers of the preparations, as given in this paper, refer to slides in the University of Chicago paleobotanical collections.

Description of plants

FILICALES

Notoschizaea robusta gen. et sp. nov. (preparations 549–553)

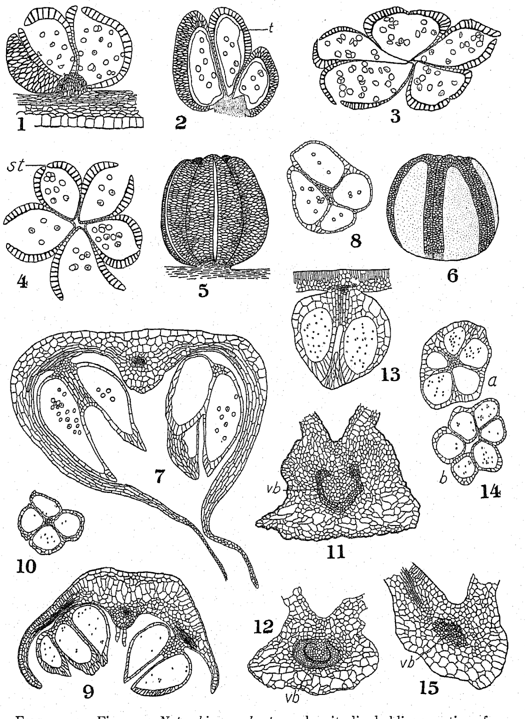
GENERIC DIAGNOSIS.—Sorus consisting of a circular group of sporangia which are so closely crowded as to resemble a synangium. Sporangia sessile, attached to the abaxial surface of a leaf by a narrow base, all of the sporangia of a sorus arising at a common point. Sporangia dehisce by means of a longitudinal cleft in the abaxial wall of the sporangium along a definite line (stomium). The outer

wall of the sporangium consists of heavy walled cells which form a wide annulus each side of the stomium.

SPECIFIC DIAGNOSIS.—Sporangia ovoid in shape, measuring about 1 mm. in length by 0.5 mm. in breadth and arranged in groups of five. Spores spherical, 55 μ in diameter, surface smooth and marked with a triradiate ridge.

This species is represented by preparations 549 to 553 in the University of Chicago collections. In preparations 551 and 552 there are excellent transverse sections of a sorus which has been slightly flattened as a result of pressure (figs. 3, 26). Five sporangia arranged in a circle make up the sorus. The sporangia are free from one another at the level of these sections but their lateral walls are flattened and in close contact. They measure about 0.5 mm. in diameter. The adjacent walls of the sporangia are thin but the outer walls are greatly thickened, measuring about 75 μ in thickness. There is a break in the continuity of the sporangium wall down the abaxial side, where dehiscence took place by means of a longitudinal cleft. On each side of this cleft the first cell (lip cell) is triangular in section, and is shorter in radial dimension than the other cells of the annulus. The apices of the triangular cells meet along the stomium. The whole of the abaxial wall of the sporangium is thus specialized to form an annulus (or more correctly, two annuli), one on each side of the stomium. The cells of the annulus are of the typical structure, with thickened inner and radial walls and a thin outer wall. Dehiscence is accomplished by the springing outward of the two annuli, thus opening a wide cleft along the stomium (fig. 4).

Preparations 552 and 553 contain obliquely longitudinal sections of the sori (figs. 1, 2). The sporangia are borne on the lower surface of a leaf and are attached thereto by means of a small common pad at their base. The sporangia are free from one another nearly to their bases. In 552 the sporangia have separated but those in 553 are closely appressed. This same state is shown in the transverse sections (figs. 3, 26) and it appears to be the normal position. In tangential section the cells of the sporangial wall are seen to be lozenge-shaped, with the long diagonal transverse to the axis of the sporangium. The cells measure about 70 by 30 μ. There appears to have been a well marked tapetum present which is preserved as a



FIGS. 1–15.—Figs. 1–5, *Notoschizaea robusta:* 1, longitudinal oblique section of sorus attached to leaf lamina (552b); 2, longitudinal but somewhat tangential section of sorus (*t*, tapetum) (553); 3, transverse section of sorus (552a); 4, same of sorus restored. Two sporangia dehiscing (*st*, stomium); 5, restoration of sorus. ×22. Fig. 6, *Corynepteris coralloides* (after ZEILLER) for comparison with *Sphaerotheca robusta.* ×20. Fig. 7, *Scolecopteris latifolia*, cross-section of fertile pinnule bearing sporangia. Synangium on left is in median longitudinal section. ×22. Fig. 8, cross-section of synangium of same near base. ×22. Figs. 9–12, *S. minor:* 9, cross-section of fertile pinnule (523); 10, cross-section of synangium (520); 11, 12, cross-sections of ultimate rachis (*vb*, vascular bundle) (520, 524). ×22. Figs. 13–15, *Cyathotrachus bulbaceus:* 13, longitudinal section of synangium (541); 14, cross-sections of two synangia (sectioned *a*, near base; *b*, near summit) (540); 15, cross-section of ultimate rachis (540). ×22.

dark line slightly separated from the sporangium wall (fig. 2 *t*). The structure of the leaf upon which these sporangia are borne is not well preserved, but the upper epidermis is very thick and has a palisade-like structure.

The spores are spherical and measure about 50 μ in diameter. The surface is marked by a triradiate ridge, but is otherwise smooth.

Having regard to the abaxial or dorsal dehiscence and to the robust sporangia, the name *Notoschizaea robusta* is proposed.

The individual sporangia of *Notoschizaea*, as far as the annulus is concerned, are much like those of *Etapteris*, in which there is a double, multiseriate, vertical annulus. The sporangia, however, are grouped in a circle in such a way as to resemble the synangium of *Scolecopteris* and related genera. *Notoschizaea*, like *Corynepteris*, combines characters of the Marattiaceae with those of the Zygopteridaceae. The resemblance to *Corynepteris* is very strong, and if the annuli of a *Corynepteris* were widened so as to occupy the whole of the abaxial surface of the sporangia, and the place of dehiscence changed from the ventral surface to the line of contact between the annuli, the resultant structure would be exactly as in *Notoschizaea* (figs. 5, 6). *Notoschizaea* is therefore placed provisionally with *Corynepteris* in the Zygopteridaceae.

Scolecopteris latifolia sp. nov. (preparations 542–544)

DIAGNOSIS.—Sporangia three to four borne in pedicellate circular synangia; united laterally and attached to a central column for about half their length, free above. Outer wall of sporangium not thicker than the lateral walls. Sporangia 1.1 mm. long by 3.5 mm. in diameter. Spores 35 μ in diameter. Leaves very broad and revolute, completely inclosing the sporangia.

The sporangia are borne in circular sori, three or four to a sorus. The sori are attached above lateral veins in a double series, one row each side of the midrib. The sori are pedicellate, the sporangia of a single sorus being attached to the pinnule by a common stalk which extends up the center of the sorus for nearly half the length of the sporangia. The sporangia are attached by their inner margins to this central column. Above the column they are free from one another (figs. 7, 27).

In shape the sporangia of *Scolecopteris latifolia* are ovate with a pointed end. In cross-section they are circular, except for the flattening where two sporangia are in contact (fig. 8). They measure about 1.1 mm. in length by 0.3–0.35 mm. in diameter. They are exannulate, and the sporangial walls are all of approximately the same thickness, about 20 μ, the outer wall not being thicker than the adjacent walls as is the case in *S. elegans* and *S. minor*. The walls consist of a single layer of platelike cells which are elongated in the direction of the axis of the sporangium. Dehiscence is by a longitudinal slit along the inner margin of the sporangium. The spores are much larger than those of either of the species previously described, being about 31 μ in diameter. Apart from the triradiate markings due to formation in tetrads, there are no special surface markings.

The sporangia are attached to the abaxial surface of leaf laminae. The pinnule is much revolute, the two margins almost meeting so as to inclose the sporangia. The pinnule if unrolled would measure about 7 mm. wide. The structure of the lamina of the leaf is poorly preserved, but from the various sections portions of all regions of the leaf may be made out. There is a thick upper epidermis and a rather thin and often indistinct lower epidermis, in which, however, stomata have been recognized. The mesophyll is without a distinct palisade layer. Between the point of attachment of the sporangia and the margin of the pinnule, the mesophyll consists of flattened platelike cells. The vascular bundle of the midrib consists of a small circular bundle of tracheids surrounded by a narrow zone of disintegrated parenchyma, probably in part phloem. In the lamina below each sporangium is a mass of transfusion tissue (fig. 7). This is probably part of a lateral vein. No transfusion tissue occurs in the column which supports the sporangia.

The sporangia of this species have the characters of those of the genus, but this species is very distinct from the two hitherto described. In general size of the sporangia it resembles *S. elegans* but is slightly larger. It differs from both *S. elegans* and *S. minor* in the relatively greater development of the central column, in the lack of thickening of the outer wall of the sporangia, and in the larger spores. The greater breadth of the leaf lamina is also a feature. In the development of the central column it resembles *Acitheca*, from which it

differs in having pedicellate synangia and in the absence of the bristle-like acuminate tips to the sporangia.

On account of the broad lamina of the pinnule the name *Scolecopteris latifolia* is proposed for this species.

Scolecopteris minor Hoskins.

Fronds of the *Pecopteris* type, bearing shortly pedicellate circular sori on the lower surface of the pinnule in a double series, one row on each side of the mid-vein. Pinnules measure about 2.8 mm. long by 1.7 mm. wide. Sori are circular, composed of four or five exannulate sporangia attached to the short pedicel by their inferior-basal margins. Sporangia ovate, roughly circular in cross-section with adjacent sides slightly flattened; distal ends extended into slightly concrescent acute apices by the elongation of the sporangial wall cells. Sporangia about 0.24 mm. in diameter by 0.57 mm. in length. Wall cells of the free surface much larger with heavier walls than those of the adjacent cells. Dehiscence occurs by means of a vertical cleft on the innermost wall of the sporangium. The spores are about 18 μ in diameter. The vascular bundle of the ultimate rachis is U-shaped with the ends of the arms slightly but abruptly involute (figs. 11, 32).

From several different coal balls, sections were obtained which give the structure of the ultimate rachis of a fernlike frond with pinnules bearing fructifications. The best of the material was obtained from two coal balls, C 210 and C 211B. The fern is so similar to that described by HOSKINS (5) as to leave little doubt as to its specific identity. There are, however, three minor differences from HOSKINS' material:

1. The pinnules are of smaller size, measuring only 2.8 mm. in length by 1.7 mm. in breadth, instead of 6–7 mm. in length by 3.3 mm. in breadth.

2. There is a well developed palisade layer in the leaf; HOSKINS' specimens had but poorly developed palisade tissue.

3. There is a wide variation in the structure of the ultimate rachis. One form (fig. 12) has a comparative weak vascular bundle surrounded by a zone of extremely thin walled parenchyma. At the other extreme the vascular bundle is much more robust and is not

surrounded by tissue different in any respect from the rest of the cortex (fig. 11). It is only the first type that HOSKINS described and figured.

These differences are modifications in vegetative structures, of the sort easily induced by environmental and other factors, and as such are not trustworthy criteria for building up taxonomic distinctions. The structural differences under (1) and (2) are possibly due to differences between a shaded environment in the case of HOSKINS' specimens and an exposed environment in the case of the specimens studied by the writer. The differences described under (3) may be due to the variation in the plane of the section. A rachis sectioned nearer the base will naturally show a larger vascular bundle and may also have other structural differences.

Cyathotrachus bulbaceus sp. nov. (preparations 535–541)

DIAGNOSIS.—Sporangia four to six, attached basi-laterally to a short central column, their bases completely united to form a saucer-shaped receptacle. The sporangia are united laterally for the greater part of their length by a common wall, a deep cup being left at the summit of the synangium. Synangia pedicellate and bulblike in shape, measuring about 0.65 mm. in diameter. Spores are 18 μ in diameter.

Preparations 535 to 541 contain sporangia attached to the under surface of a leaf lamina. There are longitudinal and transverse sections of the sporangia and obliquely transverse sections of the ultimate rachis. The sporangia are united into circular synangia similar to those of *Ptychocarpus*, but differing from that genus in several important respects.

The synangia are stalked, and each consists of from four to six sporangia united laterally at their bases to a short column which extends for about one-third the length of the sporangia, and also by a common envelope similar to but less highly developed than in *Ptychocarpus*. The uppermost parts of the sporangia are free although closely appressed (fig. 14 *b*); but nearer the base they are completely fused, the individual sporangium walls being completely merged into the common wall (fig. 14 *a*).

The sporangia are round in cross-section but are somewhat flat-

tened on their adjacent walls. In longitudinal section they are ovate in outline, with bluntly pointed tips (fig. 13). There is no annulus, but the cells of the outer wall and at the tip are larger than the others. Dehiscence probably occurred along the inner margin of the sporangia, the spores being discharged into the cup-shaped space lying between the sporangia at the summit of the synangium. The sporangia measure about 0.65 mm. long by 0.3–0.35 mm. wide. The synangia average 0.65 mm. in total diameter. The spores are round, about 18 μ in diameter, and show the characteristic tetrad markings.

In cross-section the leaf is seen to consist of an upper epidermis of platelike cells, an exceptionally well developed palisade layer consisting of much elongated prismatic cells, a spongy mesophyll, and a lower epidermis in which stomata have not been recognized with certainty (fig. 13). The synangia are attached above the secondary veins of the leaf. These veins consist of several tracheids accompanied by a much larger amount of transfusion tissue. This latter also extends into the column of the synangium but does not spread into the basal receptacle.

In the ultimate rachis (fig. 15) there is a central xylem bundle whose form appears to be elliptical in shape with the long axis parallel to the plane of the frond. The position of the protoxylem points could not be distinguished, but the tracheids are smaller on the adaxial side. Surrounding the xylem bundle is a narrow zone of exceedingly thin walled parenchyma (probably phloem), and outside this is the cortex which consists of larger cells with thicker walls. The largest cells of the cortex are found on the abaxial side of the rachis.

This sporangium is obviously closely related to those of *Cyathotrachus* and *Ptychocarpus*, with which it agrees in having a common envelope to all the sporangia of a synangium. In the presence of a central cup at the summit of the synangium it resembles *Cyathotrachus altus* Watson (13). It differs from that species, however, in not having a tracheal cup developed in the basal receptacle. Instead of this, transfusion tissue occupies the short central column as in *Ptychocarpus*, in which, however, the column extends the full length of the sporangia. In the sporangia studied by the writer structural agreement is much closer to *Cyathotrachus*.

The name *Cyathotrachus bulbaceus* is proposed for the species on account of the bulblike shape of the individual synangia. The absence of the tracheal cup, however, may possibly warrant its generic separation from *Cyathotrachus*.

Relationships of the Asterotheceae

Stur (12), in his classification of Culm and Carboniferous ferns, grouped into the suborder Asterotheceae all those forms which he considered to be of Marattiaceous affinities, those in which the sporangia of a sorus are grown together into a more or less globular synangium. The suborder included four genera: *Asterotheca* Presl, *Scolecopteris* Zenker, *Sturiella* Weiss (*Renaultia* Stur), and *Ptychocarpus* Weiss (*Diplazites* Goepp.). To these four there must now be added two others. Practically all paleobotanists have placed the fructifications of *Pecopteris polymorpha* in the genus *Scolecopteris*, although Schimper (10) in 1890 founded the genus *Acitheca* for its reception. In 1925 Kidston (6) agreed with Schimper in excluding this species from the genus *Scolecopteris*, a view which in the writer's opinion is justified on the basis of structural differences. In 1906 Watson (13) described isolated synangia for which he erected the genus *Cyathotrachus*. Brief descriptions of these six genera follow.

Asterotheca.—Synangia sessile, consisting of three to eight exannulate sporangia which are more or less ovate, with the free end brought to a short acute apex. The major axis of the sporangium is usually parallel to the frond which supports it. Dehiscence is by means of a longitudinal slit along the inner margin of the sporangia.

Scolecopteris.—Sporangia united laterally at their bases into a circular synangium which is shortly pedicellate. Sporangia free above, and dehiscing by a longitudinal slit along their inner margin. The long axis of the sporangia is perpendicular to the surface of the frond. The main difference between this genus and the preceding is the presence of a pedicel to the synangium.

Acitheca.—Synangia sessile, usually formed of four (rarely three or five) sporangia. Sporangia exannulate, long, rounded at base, and terminating in a sharp bristle-like point, the upper two-thirds of their length free, the basal third united to a central column. The synangia thus formed are attached by their broad bases perpendicu-

larly to the lower surface of the pinnule. The column or receptacle into which a vascular bundle extends has four plates or wings to which the sporangia are united. Dehiscence takes place through a vertical cleft along the adaxial margin of the sporangia. The margins of the pinnules are recurved and almost cover the synangia.

Sturiella.—Sporangia in groups of five, united below by their fused bases which form a concave plate attached to the pinnule by a short pedicel. The sporangia are described as cylindrical, pyriform, with adjacent sides slightly flattened (9). There is an apical annulus which caps the sporangium and runs down the dorsal wall for some distance toward the base. Dehiscence is by means of a longitudinal cleft along the inner margin of the sporangium.

Cyathotrachus.—The synangium consists of from four to seven sporangia grouped around a central receptacle which is hollowed out into a cup above. The whole is surrounded by a continuous integument. The whole structure was attached to the organ which bore it by a small base. The base apparently received a bundle from the stalk or support, and this bundle spread out into a cup of short tracheids in the sterile tissue at the base of the synangia. The sporangia probably dehisced into the central cup.

Ptychocarpus.—Sporangia almost cylindrical, contracting slightly upward with a truncate apex, attached throughout their entire length to a central column and united to each other laterally, the individual sporangia of the synangium thus formed being inclosed in a common envelope of ground tissue. The synangia are shortly stalked and each consists of from five to eight sporangia. The mode of dehiscence is unknown.

With the exception of *Cyathotrachus*, in which the pinnules are unknown, the shape of the frond in these genera is known to be of the *Pecopteris* type. The sporangia are borne in a double series, one each side of the mid-vein, the attachment of the sporangia being directly over a lateral vein.

It seems reasonable to assume that the primitive state in the Asterotheceae was that of groups of separate sporangia, each sporangium being sessile on the leaf. The fusion of individual sporangia into a synangium having a common envelope and the development of a pedicel to the synangium in all probability represent specializa-

tions. *Ptychocarpus* represents the final product of this line of evolution. The accompanying provisional family tree (fig. 38) has been drawn to show the relations among the genera.

PTERIDOSPERMS

Telangium pygmaeum sp. nov. (preparations 545–548)

DIAGNOSIS.—Branching rachis without lamina, bearing synangia terminally or laterally on its ultimate ramifications. Synangia circular, consisting of three to five sporangia which are fused laterally for about one-third their length. Sporangia attached to the rachis by a broad base, ovoid in shape, measuring 0.6 mm. by 0.19 mm. Spores circular, 35 μ in diameter, smooth, and marked with a triradiate ridge.

Preparations 545 to 548 contain small exannulate sporangia grouped into circular synangia. These are attached to a badly preserved rachis which does not appear to be a foliage leaf (figs. 16, 30). The walls of the sporangia appear to be but one layer of cells in thickness. The sporangia are fused laterally for the basal one-third to one-half of their length. For the attachment of the terminal synangium the rachis is expanded. Other synangia are attached laterally to the same rachis by a broad area of attachment. The synangia are distinctly sessile.

Digitate clusters attached to branching petioles devoid of lamina, and associated with or attached to leaves of the *Sphenopteris* type, have been known for a great many years as plant impressions from Paleozoic rocks. These were first investigated by STUR (11) who gave to them the name *Calymmatotheca*. *C. stangeri* is the type species of the genus. STUR considered the constituent parts of the digitate clusters as indusial bracts, but RENAULT considered them to be sporangia.

These two conflicting opinions were held until BENSON (1) obtained petrifactions of sporangia which led her to make a careful reinvestigation of STUR'S type specimens of *Calymmatotheca*. She concluded that STUR'S interpretation as to the nature of his specimens was correct, and that the sporangia would have to be excluded from that genus. The form genus *Telangium* with *T. scotti* as the type species was erected for these sporangia.

BENSON (1) gives the following diagnosis for the genus *Telangium:*

Fertile and barren pinnae dissimilar; fertile pinnae represented by synangia only; synangia borne at the extremity of the ultimate ramifications of the rachis, composed of six to twelve sporangia which taper to the apex and are united primarily for almost their whole length to form a body which is continued into a sterile base of decreasing diameter through which runs longitudinally a single vascular strand. Each sporange ultimately becomes almost free from the others by septicidal dehiscence and liberates large spores from a ventral suture.

One species with structure preserved, *T. scotti*, was described by BENSON. Several other species, based upon impressions only, have also been placed in this genus.

The following is a list of the previously known species:

T. scotti Benson, from the Gannister beds of Lancashire.

T. affine L. & H. and *T. bifidum* L. & H., both from the Calciferous Sandstone series of England.

T. digitatum Kidston from the Lanarkian of England.

T. nutans Carpentier from the Upper Carboniferous of France.

The fertile frond of *Sphenopteris potieri* may prove to be a *Telangium*. It is found in the Upper Carboniferous of France and also from the Staffordian of England. *T. asteroides* Lesq. has proved to be an imperfect specimen of *Crossotheca hoeningshausii*, and must therefore be dropped from the list of species of *Telangium.*

As the Illinois specimens are very much smaller than any of those previously described, the name *Telangium pygmaeum* is proposed. *T. pygmaeum* is sharply separated from the other species of *Telangium* both by its smaller size and also by the smaller number of sporangia making up a synangium. The sporangia measure only a little more than 0.5 mm. in length, whereas *T. affine*, the smallest of the previously described species, measures 2.5–3. mm., and *T. digitatum*, the largest, has a synangium composed of about 25 sporangia which attain a length of 8 mm. *T. pygmaeum* occurs considerably later in geologic time than the other species.

Conostoma platyspermum sp. nov. (preparations 501–516)

DIAGNOSIS.—Seed platyspermic, ribs absent, seed sometimes slightly 2-angled. Integument and nucellus undiverged at plinth level. Vascular strands two, in the inner region of the integument, each corresponding to an angle of the seed. Size ranges up to 4.5 mm. in length by 2.5 mm. in width by 2.0 mm. in thickness.

The genus *Conostoma* was founded by WILLIAMSON (14) in 1887. It included three species of small Paleozoic seeds: *C. oblongum* from the Gannister beds of the Lancashire coal fields, and *C. ovale* and *C. intermedium* from the Calciferous Sandstone series of Burntisland, Scotland. The latter two species were re-investigated by BENSON, however, who reduced them to one; and, in view of its structural differences, removed it from the genus *Conostoma* and made it the type of a new genus, *Sphaerostoma*. In 1911 OLIVER and SALISBURY (8), having new and more abundant material available, restudied *Conostoma*. *C. oblongum* was redescribed in greater detail, and a new species, *C. anglo-germanicum*, was founded on material from Shore, Littleborough, England, and from Duisburg in Rheinpreussen, Germany. KRICK (7) has also described *C. oblongum* from coal ball material from coal no. 5 at Harrisburg, Illinois.

In the study of coal ball material from Calhoun, a related but undescribed species of seed came to light. The seed is represented by a number of specimens, all found in the same coal ball, but without any organic connection between them and any other material. In view of the pronounced platyspermic nature of the seed it is proposed that the new species be called *Conostoma platyspermum*.

OLIVER and SALISBURY (8) give the following diagnosis of the genus *Conostoma:*

> Cylindrical or slightly flattened seeds with tapering insertion. Ribbed throughout, or at base with angles passing into ribs. Lobing at apex variable; vascular bundles equalling or fewer than the ribs or angles; loculi of canopy nearly obliterated and equalling the vascular bundles in number; epidermis mucilaginous. Lagenostome very small, included; cells of wall sculptured. Plinth conspicuous, dome shaped, with internal tissue; well marked "tent pole" and tapetum present.

The Illinois specimens agree in all essential features with this generic diagnosis, and are thus included in the genus *Conostoma*.

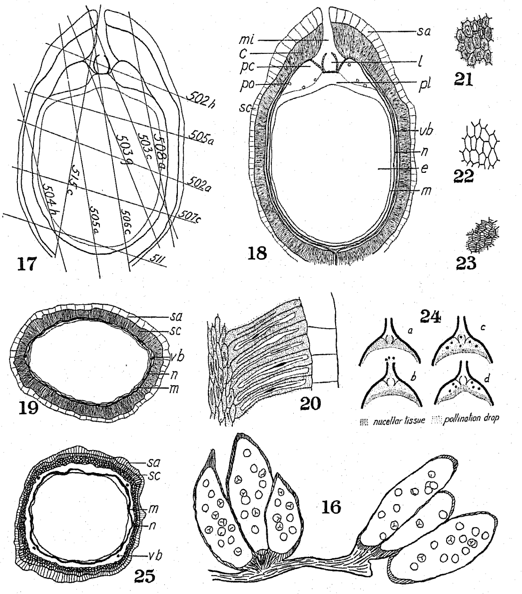
The new species differs from *Conostoma oblongum* and *C. anglo-germanicum* in being distinctly platyspermic, although *C. oblongum* in some measure shows this feature. In cross-section the seed is elliptical in outline. In all three of the specimens of which transverse sections are at hand (preparations 502, 505–512, 513) the flattening is parallel to the plane in which the two vascular bundles lie (principal plane of the seed), so that such flattening can scarcely be ascribed

to deformation of the seed (fig. 19). In longitudinal section the seed is somewhat oblong, with more or less parallel sides (figs. 18, 32, 33), but the whole structure is relatively shorter than that of *C. oblongum* and *C. anglo-germanicum*. An interior mold of the seed is shown in figure 31.

The new species has a single integument and the nucellus is undiverged to near the summit of the latter. The integument has a characteristic structure, making it easy to recognize various sections of the seed as belonging to the same species. It is composed of three distinct layers: a sarcotesta; a middle layer or sclerotesta; and an inner layer of delicate tissue, for the most part not preserved, but which must have surrounded the nucellus and in which the vascular bundles ran. In some cases the greater part of the thin sarcotesta has been lost by exfoliation, from which habit OLIVER and SALISBURY gave to it the name "blow-off" layer in their description of *C. oblongum*. The sclerotesta is variable in thickness, depending on the seed and on the plane of section. In the body of the seed its thickness is 0.1–0.23 mm. At the summit the integument becomes much thickened to form a conical canopy surrounding the micropyle.

The nucellus measures about three-fourths the length of the seed. It is non-diverged from the integument for its whole length (fig. 18). At the summit there is a large pit or depression, from the floor of which the small lagenostome arises. The lower portion of the nucellus is replaced by the growth of the megagametophyte (endosperm), the megaspore membrane marking the contact of the two structures. Only faint and indefinite traces of nucellar and megagametophyte tissues are preserved, excepting the tapetal layer of the nucellus surrounding the latter. The part of the nucellus above the megagametophyte is known as the plinth. The summit of the plinth is occupied by a plinth cavity which functioned as a pollen chamber.

The hard part of the testa which gives the shape to the seed is elliptical in cross-section, cylindrical, and tapering with a steep curve toward the micropyle, and curving more sharply to the chalaza at the other. Thus the longitudinal section of the seed has the characteristic boat shape of *Conostoma*, the micropylar end representing the prow and the chalaza the stern. The length of the seed varies from 1.6 to 2.6 mm. in the measured longitudinal sections; but if the seed



Figs. 16–25.—Fig. 16, *Telangium pygmaeum:* two synangia attached to a rachis (546). ×44. Figs. 17–24, *Conostoma platyspermum:* 17, diagrammatic sketch of median longitudinal section of seed plotting approximate positions of chief sections used in this paper; 18, reconstructed longitudinal section (*c*, canopy; *e*, megagametophyte [endosperm]; *l*, lagenostome; *m*, megaspore membrane; *mi*, micropyle; *n*, tapetal layer of nucellus; *pc*, plinth cavity or pollen chamber; *pl*, plinth; *po*, pollen grains; *sa*, sarcotesta; *sc*, sclerotesta; *vb*, vascular bundle). ×11; 19, cross-section (508a). ×11; 20, semi-diagrammatic drawing of radial section of testa showing sarcotesta and the two zones composing sclerotesta. Note shrunken protoplasm in prismatic cells (514). ×93; 21, tangential section of same. ×93; 22, tangential section of epidermis of plinth (515c). ×93; 23, tangential section of wall of lagenostome showing sculptured cells (506). ×93; 24, diagrammatic representation of summit of seed showing development of pollen chamber and method of pollination. Fig. 25, cross-section of seed of *C. quadratum* (518b), legend as in fig. 18. ×22.

shown in cross-section in figures 19 and 49 has the same relative proportions, its length must be about 4.5 mm. In transverse section the seeds show their platyspermic nature. Preparation 502 (fig. 34) measures 1.5 by 0.9 mm., maximum and minimum diameters, and the other specimen (507c, fig. 19) measures 2.5 by 2.0 mm. In both cases the flattening was in the plane of the seed which includes the two vascular bundles. In the first specimen the sclerotesta is somewhat thickened above the vascular bundles, but the other shows no indication of increased thickness. In no case were there any ridges in the testa such as is characteristic of *C. oblongum* and *C. anglo-germanicum*.

The internal form of the testa is, in the main body of the seed, similar to the exterior. The summit is dome-shaped, however, and passes into the funnel-like lower end of the micropyle. At the apical end of the seed the testa is markedly thickened to form the canopy.

The integument consists of three distinct zones. From the exterior inward they are: the sarcotesta, the sclerotesta, and a delicate thin walled zone through which the vascular bundles progress. The sarcotesta consists of a single layer of light colored cells investing the external surface of the seed. At the apex the cells of this layer are much enlarged to form a fleshy cap to the seed. The length of these cells is 160 μ, which is in contrast to the thickness of about 40 μ for the sarcotesta of the remainder of the seed.

There is some doubt as to the true shape of the apical end of the seed. In two specimens (503–504 and 505–512) the seed appears to be truncated abruptly, the enlarged epidermal cells forming a cylinder surrounding a wide and squarely truncated canopy (figs. 32, 35). In 515c (fig. 33) the canopy appears to be of the more nearly normal dome shape, however, with the sarcotesta reaching to its summit and surrounding the micropyle. The writer is inclined to the view that the truncated appearance of the other specimens is due to destruction of tissue prior to fossilization. He has therefore shown a complete sarcotestal cap in his reconstruction (fig. 18).

As its name implies, the sclerotesta is made up of sclerotic, thick walled elements, its thickness varying from 0.1 to 0.23 mm. in various seeds. There are two distinct layers of tissue making up the sclerotesta (fig. 20). The outer layer consists of a single series of

palisade cells, the inner of elongated sclerotic cells disposed longitudinally. The amount of this tissue is variable; in some cases the palisade layer constitutes most of the integument, in others the longitudinally elongated elements make up one-third of the thickness. The cells of the prismatic layer are roughly hexagonal in cross-section (fig. 21). The cells are extremely thick walled and have a narrow lumen. The shriveled protoplasmic contents (fig. 20) show as black specks, and give to the integument, as seen in section, a peculiar peppered appearance.

A single vascular bundle enters the chalazal end of the seed, and after penetrating to the inner layers of the integument, divides into two. These branches are in the plane of the major diameter of the seed, and lie in the inner layers of the integument, just outside the nucellus. Whether they extend into the canopy could not be determined from the specimens, but by analogy with *Conostoma oblongum* it is probable that they do. As seen in cross-section, the individual bundle consists of about eight or nine tracheids exhibiting scalariform thickenings (fig. 34).

The nucellus falls into three regions: (1) the lagenostome, which arises from the base of a cuplike depression at the summit of (2) the plinth or distal portion of the nucellus, and (3) the main part of the nucellus, now almost completely replaced by the growth of the megagametophyte within it. The nucellus is undiverged from the integument except for the cuplike depression at the summit of the plinth, and for the lagenostome. This differs from the two previously described species in which the nucellus was free from the integument at the level of the plinth.

The body of the nucellus has been almost completely replaced by the growth of the megagametophyte, whose limits are marked by a brown line representing the megaspore membrane. Only in one specimen (section 514) is there any gametophyte tissue preserved, and in this the preservation is extremely poor. All that can be distinguished are a few delicate parenchyma cells. Of the nucellus only the tapetal layer remains, and this is crowded between the megagametophyte and the integument. This tapetal layer consists of extremely large flattened cells of polygonal outline (fig. 36), measuring about 150 μ in diameter by 40 μ in thickness.

LAGENOSTOME AND PLINTH.

These two organs, which form the summit of the nucellus, are so intimately related that they may most conveniently be dealt with together. The distal portion of the plinth is occupied by the plinth cavity (fig. 18 *pc*). The proximal portion of the plinth was occupied by nucellar tissue. With the exception of a plug of crushed tissue at the base of the lagenostome, none of this tissue is preserved, but the boundary between this and the plinth chamber is marked by a faint brown line. Apart from these remains of internal filling tissue, the plinth is represented only by the epidermis which, where its structure is well enough preserved, is seen to consist of a single layer of flattened epidermal cells without special sculpturing (fig. 22).

Following the epidermis of the plinth down the slope of the cup at its summit, it becomes evaginated to form the lagenostome. Thus the epidermis of the plinth and of the lagenostome is one continuous structure. The lagenostome, as in *Conostoma oblongum*, is a tiny goblet-shaped body, the cavity of which communicated with the plinth cavity at the time of pollination. Unlike *Physostoma* and *Lagenostoma*, the mouth is unprovided with any tube or beak. Although none of the specimens studied shows it, by analogy it seems probable that the mouth engaged with the micropylar tube by means of a bevelled flange such as is found in *C. oblongum*. The lagenostome measures 170 μ in maximum diameter by about 150 μ in height (fig. 35). The wall of the lagenostome is formed of a single layer of cells which have an elaborate tracheid-like sculpturing (fig. 23). The floor of the lagenostome does not consist of differentiated sculptured cells but is occupied by a pad of crushed nucellar tissue, the individual cells of which are for the most part indistinguishable. The formation of this pad will be discussed later in connection with the method of pollination. The plinth chamber (fig. 18) lies at the very summit of the nucellus. It is annular in shape and approximately triangular in cross-section. It is bounded on the inside by the epidermis of the depressed cup at the summit of the nucellus; on the outside by the undiverged nucellus and integument; and below by nucellar tissue. This plinth cavity functioned as a pollen chamber, and it is within this cavity that the pollen is nearly always found. In only one case (fig. 3) was any pollen found in the lagenostome.

The pollen grains are multicellular, elliptical in form, and measure 50 by 65 μ in diameter. In common with other Paleozoic seeds there is no evidence of the development of pollen tubes. The absence of pollen tubes and of embryos in Paleozoic seeds may possibly both be due to the same cause, namely, that the "seeds" so far studied are really ovules. The fact that the integument is made up of thick walled stony cells is not incompatible with this view, since in modern gymnosperms, as in the cycads and some conifers, the integument may have become sclerotic before the archegonia are developed.

An outline of the probable course of events in the history of ovular maturation and pollination will help in understanding the relationships of the various parts. At an earlier stage of development than any represented by the preparations, the tip of the nucellus must have been occupied by a soft internal tissue which filled the lagenostome and the plinth, the tissue being continuous from one to the other through the narrow orifice by which these structures communicated (fig. 24 *a*). As the time of pollination drew near, the filling tissue of the lagenostome and the summit of the plinth doubtless underwent degeneration (fig. 24 *b*). A pollination drop was exuded at this time. It was probably formed in part by the mucilaginous substances resulting from disintegration of the internal tissues of the lagenostome and plinth, and in part by secretion from the tracheid-like sculptured cells which make up the walls of the lagenostome. The pollen was caught by this exuded drop, which, as it dried, carried the pollen grains through the lagenostome into the plinth chamber (fig. 24 *c*). The plinth cavity was then closed by the growth of the "tent-pole" of the megagametophyte. This forced the overlying nucellar tissues against the base of the lagenostome, thus sealing the opening (fig. 24 *d*).

Two species have previously been described, *Conostoma oblongum* and *C. anglo-germanicum*. *C. platyspermum* shows more features in common with the former species. In its general proportions and the relation of its height to width, it bears a much stronger resemblance to the former; indeed, in proportion to its length it is even broader than that species. Also, in its platyspermy and in the absence of ridges or wings it resembles *C. oblongum* more closely. In the reduced number of vascular bundles, however, it shows agree-

ment with *C. anglo-germanicum*, and with *Gnetopsis elliptica*, another seed belonging to the *Conostoma* group. In view of the platyspermic nature of *C. oblongum*, *C. platyspermum*, and *Gnetopsis elliptica*, it is interesting to note that in *C. oblongum* there are six vascular bundles, one beneath each of the two major angles of the seed and four intermediate bundles. In *Gnetopsis* the bundles corresponding to the major angles have been lost. In *C. platyspermum*, on the other hand, only the two bundles corresponding to the major angles of the seed are present.

Conostoma quadratum sp. nov. (preparations 517–519)

DIAGNOSIS.—Seed squarish in cross-section. Ribs absent. Radispermic with four vascular bundles. Diameter 1.35 mm.

A number of closely spaced transverse sections of a small seed were obtained by the film method from coal ball C 237. Only the basal portion of the seed is known, as the apical portion was lost in the primary saw cut.

The seed is extremely small, measuring only 1.35 mm. in diameter. The general arrangement of its tissues (figs. 25, 37) is that of a member of the *Conostoma* group. The integument consists of a sarcotesta comprising a single layer of epidermal cells, and a sclerotesta comprising an outer prismatic zone and an inner non-prismatic zone. Four vascular bundles, each corresponding to a corner of the seed, run longitudinally in the now empty space between the integument and nucellus. In the cross-sections the nucellus appears to be free from the integument, owing partly to shrinkage and partly to disintegration of the delicate tissue which formed the innermost part of the integument. The loss of tissue makes it appear as if the nucellus were free from the integument. The same appearance is seen in cross-sections of *C. platyspermum* but in that case the longitudinal sections leave no doubt at the non-divergence of the nucellus and integument. The limits of the megagametophyte are defined by the megaspore membrane. The testa is variable in thickness, measuring 90–180 μ. This variation bears no relation to the vascular strands nor to the symmetry of the seed (figs. 25, 37).

The sarcotesta is relatively more extensive than in the other species of *Conostoma*, as it makes up one-third to one-half the thickness

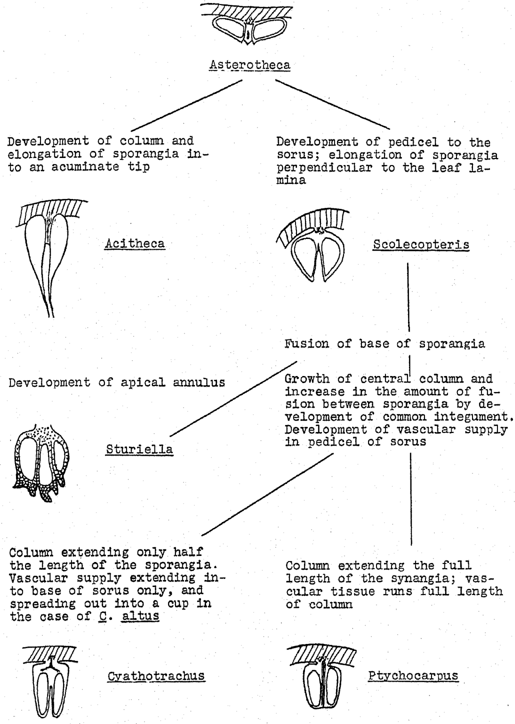


FIG. 38.—Provisional family tree showing relations among genera of the Asterotheceae.

of the testa, and there appears to be no tendency for it to be exfoliated. The sclerotesta consists of two zones, a prismatic zone on the outside and a non-prismatic one on the inside. The prismatic zone is made up of a single layer of stout cells averaging 20 μ in diameter and 40–50 μ in length. The inner zone of the sclerotesta is made up of what appears in transverse section to be isodiametric thick walled cells. By analogy with those of closely related seeds, these cells probably are really longitudinally elongated.

The four vascular bundles are found at the corners of the seed in the empty space lying between the sclerotesta and the nucellus. In some places there are slight traces of a delicate parenchyma which formerly occupied this space. Each vascular bundle consists of about twelve minute tracheids, approximately 3 μ in diameter. The type of tracheal sculpturing could not be resolved very well but it appeared to be scalariform. The sections at the chalaza show that a single vascular bundle enters the seed and passes into the inner layers of the integument, where it divides to give rise to the four bundles.

Only the tapetal layer of the nucellus is preserved. It consists, as in *Conostoma platyspermum*, of the large platelike polygonal cells. The megaspore membrane is for the most part in contact with this layer. Where this is not the case, the condition is probably due to shrinkage. No endosperm is preserved.

The undiverged nucellus, and the fact that the vascular bundles run in the innermost region of the integument, place this seed in the group Lagenostomales. Unfortunately micropylar sections are lacking, so that the most characteristic structures on which the classification of this group is based are absent. However, the structure of the integument is that typical of the Conostomae (7). In this group there are but two genera, *Conostoma* and *Gnetopsis*, and the similarity of the latter genus to the former may possibly warrant its inclusion in *Conostoma*. In view of this fact, this seed is assigned there provisionally. The discovery of other specimens showing the micropyle and lagenostome will of course be necessary to confirm this classification. Having regard to the squarish cross-section of the seed, the name *Conostoma quadratum* is proposed.

As far as limited material permits one to say, *C. quadratum* agrees

more closely with *C. anglo-germanicum* than with any other species. In common with that species, the sarcotesta is more extensive and persistent than in the other species. It also agrees in having radial symmetry and four vascular bundles. It differs markedly from that species in the entire absence of ribs, and it is much smaller in size. In the absence of ribs it shows agreement with *C. platyspermum*, from which, however, it differs markedly in other respects, as in general shape of the seed and in the histological structure of the integument (*cf.* figs. 19 and 25).

Summary

1. A morphological study was made of plant material preserved in coal balls from Calhoun coal mine, Richland County, Illinois. The geologic horizon is Middle Conemaugh, early Upper Pennsylvanian.

2. Preparations were made by the cellulose peel method.

3. Seven plants are described in detail. Of these, four are sporangia of ferns, one is the microsporangium of a pteridosperm, and two are seeds. The plants described are: **Notoschizaea robusta** gen. et sp. nov., **Scolecopteris latifolia** sp. ncv., *Scolecopteris minor* Hoskins, **Cyathotrachus bulbaceus** sp. nov., **Telangium pygmaeum** sp. nov., **Conostoma platyspermum** sp. nov., and **Conostoma quadratum** sp. nov.

4. A short discussion of the ferns belonging to the Asterotheceae is given, with a phylogenetic tree showing probable relationships.

5. It is suggested that the absence of tubes in the pollen found in the pollen chambers of Paleozoic seeds and the absence of embryos may possibly be due to the same cause, namely, the fact that the so-called seeds are really immature ovules.

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EXPLANATION OF PLATES VIII, IX

PLATE VIII

FIG. 26. *Notoschizaea robusta:* transverse section of sorus (552a). ×50.

FIG. 27. *Scolecopteris latifolia:* cross-section of pinnule (543b). ×37.

FIG. 28. *Cyathotrachus bulbaceus:* transverse section of synangium (540). ×55.

FIG. 29. Longitudinal section of synangium of same (541c). ×55.

FIG. 30. *Telangium pygmaeum:* two synangia in longitudinal section attached to rachis; other synangia cut obliquely (546). ×45.

FIG. 31. *Conostoma platyspermum:* internal cast of seed (*pl*, plinth; *tp*, "tent-pole" or beak of endosperm) (501). ×33.

PLATE IX

Conostoma platyspermum

FIG. 32. Obliquely longitudinal section of seed (503f). ×30.

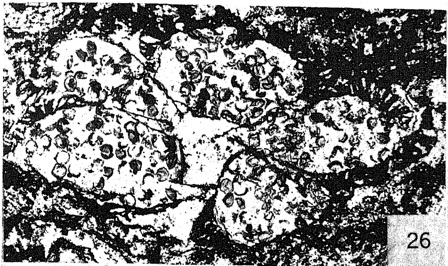
FIG. 33. Same showing complete canopy (515). ×30.

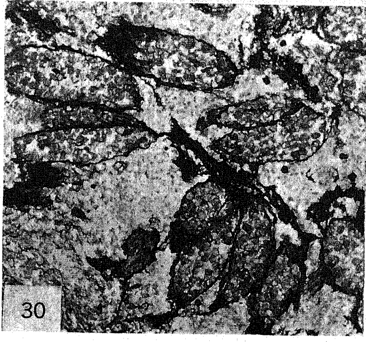
FIG. 34. Transverse section showing vascular bundles (*vb*) (502b). ×33.

FIG. 35. Longitudinal section of apical portion of seed (*i*, integument; *l*, lagenostome; *mi*, micropyle; *mm*, megaspore membrane; *pc*, plinth cavity) (506c). ×60.

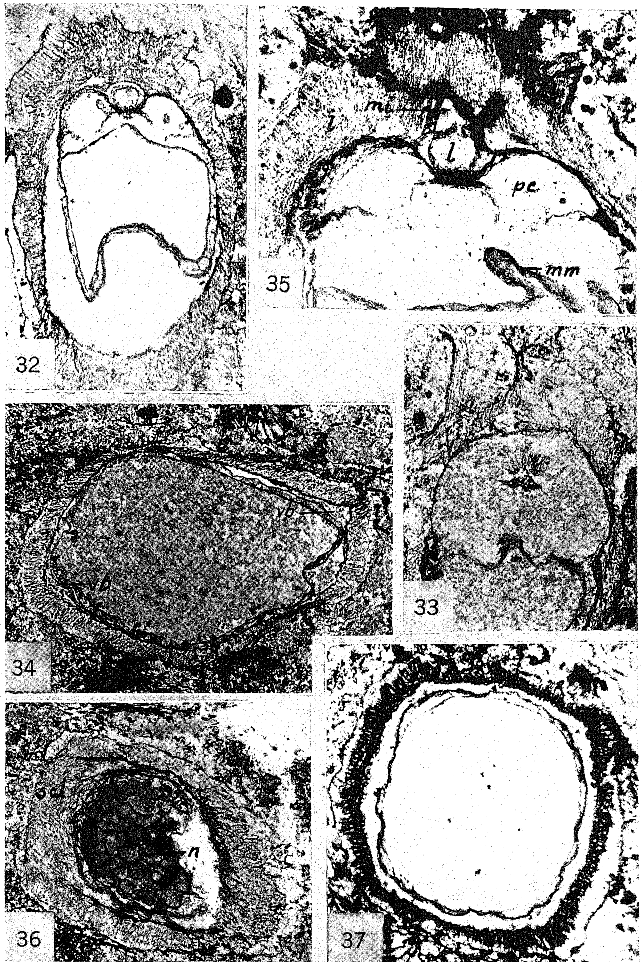
Fig. 36. Tangential section showing sclerotesta (*scl*) and tapetal layer of nucellus (*n*) (504h). ×30.

FIG. 37. *Conostoma quadratum:* cross-section of seed. ×50.





GRAHAM on PENNSYLVANIAN FLORA



GRAHAM on PENNSYLVANIAN FLORA

MALE GAMETOPHYTE OF ATRIPLEX HYMENELYTRA

FREDERICK H. BILLINGS

(WITH ELEVEN FIGURES)

Introduction

Atriplex hymenelytra is a low statured, dioecious shrub which grows in certain parts of the Mojave and Colorado deserts in southern California. Because of its holly-like leaves it is popularly known as desert holly, or silver holly, the latter name having reference to its white leaves. *Atriplex* is represented in California by both monoecious and dioecious species, numbering twenty-nine in all according to JEPSON (4). Ten of these species are reported as dioecious.

The chief purpose in undertaking a study of this species was to ascertain the type of chromosome sex mechanism. The material on which the observations were based was gathered near Barstow on the Mojave desert, in February and March, at which time of year winter rains are generally sufficient to enable shrubs to put forth a new crop of leaves and flowers. Young buds were fixed in Carnoy's fluid, mounted in rows on cardboard squares, imbedded in nitrocellulose and sectioned on a sliding microtome. Iron-alum haematoxylin was used as a stain.

Observations

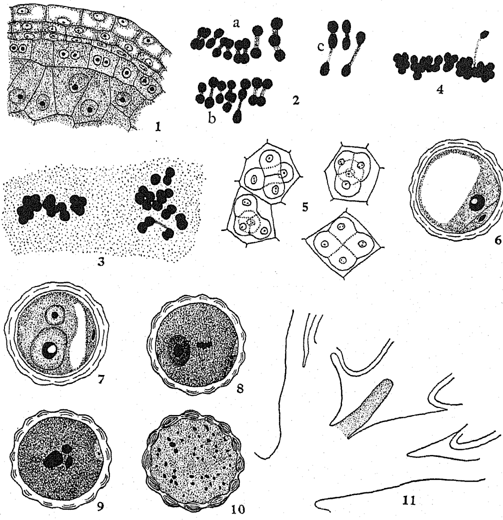
The archesporium arises from hypodermal cells which through periclinal divisions differentiate an inner sporogenous tissue and an outer parietal layer. The maturity of the pollen mother cells is approximately contemporaneous with the completion in development of a tapetum, which is composed mainly of binucleate cells with rich cytoplasmic contents (fig. 1). Meiosis occurs simultaneously in the mother cells of any single anther. The chromosomes that arrange themselves on the metaphase plate are short and slightly elliptical in outline. The process of division is normal, that is, without lagging chromosomes or extruded chromatin. Nine was found to be the haploid number in some of the daughter cells, ten in others, the variation

depending on whether the cell inherited a Y-chromosome along with the eight autosomes, or a bipartite X. Nine appears to be a common N number in the Chenopodiaceae, as may be seen from the following list of species:

SPECIES	N	AUTHORITY
Chenopodium album...	9	WINGE
C. hybridum..........	9	"
C. murale..........	9	"
C. vulvaria..........	9	"
C. bonus henricus......	18	"
Atriplex hastatum......	9	"
A. litorale............	9	"
A. patulum............	18	"
Beta maritima.........	9	"
Bassia hirsuta......	9	"
Hablitzia tamnoides....	9	DAHLGREN
Spinacea oleracea......	6	WINGE

In *Atriplex hymenelytra*, one of the chromosomes that appears on the metaphase plate in the heterotypic division is not a continuous structure, but is tripartite (fig. 2 *a*). In this particular meiotic figure it stood well away from the remaining eight so that an unobstructed view was obtained. The individual elements were approximately equidistant from one another, with no indication as to which one would separate from the other two. The elements were globose in form and about equal in size, the entire tripartite structure measuring 3.1 micromillimeters in length. To all appearances there were three separate and distinct chromosomes, all of about the same size and form as the other chromosomes. In anaphase, a terminal one separates from the other two, which then draw closer together. The best views of this stage were obtainable when a portion of the chromosome group was cut away (fig. 2 *b*, *c*). The next stage observed was one in which a double member was seen at one of the poles (fig. 3). Counting the bipartite member here seen as two separate chromosomes, the number was found to be ten. It is apparent that there is a partial fusion of the elements after they have reached the poles.

A homoeotypic division is represented in figure 3, in which a polar view of one metaphase plate is obtained and a side view of the other.



FIGS. 1–11.—Fig. 1, pollen mother cells and binucleate tapetum just before meiosis. ×400. Fig. 2, heterotypic metaphase (*a*, 8 autosome bivalents and a tripartite X–Y group with elements equidistant from one another; *b*, *c*, incomplete chromosome groups, but ones in which disjunction of Y from a bipartite X is clearly indicated). ×2700. Fig. 3, homoeotypic metaphase. One chromosome group shows separation of the daughter bipartite X's with an N count of 10; the other group, probably containing the Y, gives an N count of 9. ×2700. Fig. 4, somatic metaphase taken from cell in anther wall; 18 chromosomes. ×2700. Fig. 5, diagrams illustrating various groupings of microspore tetrads. Fig. 6, vacuolated 2-celled male gametophyte with one cell walled off, and resembling a prothallial cell. ×700. Fig. 7, 3-celled male gametophyte. Tube and generative nuclei free in main cavity. ×700. Fig. 8, 3-celled gametophyte showing division of generative nucleus. Nucleus of walled-off cell undergoing disintegration. ×700. Fig. 9, mature 4-celled gametophyte. ×700. Fig. 10, mature pollen grain showing complete nuclear disintegration. ×700. Fig. 11, diagram of base of a staminate flower at time of anthesis. Gynoecium reduced to a sterile column of tissue. ×42.

Examination of the chromosomes shows that there is a double member on one plate but not on the other. Separation of the bipartite daughter chromosomes has proceeded in advance of the others.

A somatic division obtained from one of the cells in an anther wall is represented in figure 4. No bipartite chromosome was observed here, so it was inferred that it is an element in the chromosome complement of the pistillate plant. Precocious separation of a single pair is shown.

In both plants and animals in which a tripartite chromosome appears in the heterotypic division, the structure has been interpreted by certain investigators as consisting of three chromosomes, in which case an extra chromosome is allotted the daughter nucleus receiving the bipartite X. For example, MEURMAN (6) found in *Rumex acetosella* an X-Y type which resembles that found in *A. hymenelytra*, in that it is tripartite. Apparently it differs, however, in that the X is constricted only and is not divisible into two separate elements in an earlier state. The middle segment in *R. acetosella* is considerably the largest of the three. In anaphase the Y disjoins from the constricted X. In polar view of the homoeotypic metaphase plate, the X member can be seen as bipartite. In *A. hymenelytra* the three elements of the tripartite X-Y complex are approximately the same size. It would seem to be a matter of minor importance whether the elements are of equal size or not. In *R. acetosella* the daughter nucleus receiving the constricted X is reported as having a total count of twenty-one (nineteen autosomes and the X which is counted as two); whereas the other daughter nucleus, carrying nineteen autosomes and the Y, is credited with twenty. In *R. acetosa*, the tripartite structure as seen by KIHARA and ONO (5) varies in its behavior from *R. acetosella* and *A. hymenelytra*, in that both terminal segments pass to the same pole at disjunction, leaving the large middle segment to pass to the other pole. In this instance there is a complete separation of all three portions of the original tripartite chromosome complex, the nucleus receiving the end segments having a count of one more than that receiving the middle member. WILSON (10) regards the two small chromosomes as elements in a Y complex. *R. acetosa* resembles *A. hymenelytra* in possessing three separate and distinct elements as the heterochromosome group.

Interestingly enough, an X-Y system closely resembling that in *A. hymenelytra* is found in the opossum (*Didelphys virginiana*), as reported by PAINTER (7). The X chromosome in this animal is composed of a pair of similar egg-shaped elements which appear together, not only in the maturation divisions but also in the somatic divisions in the female. PAINTER, however, regards the X member as a single chromosome and consequently assigns an equal chromosome count to each daughter nucleus, that is, eleven in the maturation divisions and twenty-two in the somatic divisions of both males and females. WILSON gives a list of insects in which the X is a double member which he regards as two chromosomes, thus giving one half the sperm nuclei an excess of one in the chromosome count. In a species in which the X (or Y) is seen at some time to consist of two separate and distinct elements, there are good reasons for considering such a member as two, even though the elements are in conjunction or partial fusion at another time. On the other hand, one might be tempted to regard a merely constricted chromosome as single were it not for the presence of intergrading or borderline instances in which the constriction becomes so deep or so long as virtually to differentiate two chromosomes. This appears to be the case in the opossum, as shown by some of PAINTER's representations of the X. In *A. hymenelytra*, however, the situation seems fairly clear, inasmuch as three separate and distinct elements, one the Y, appear in metaphase, the X element soon partially fusing to form a constricted chromosome. The N chromosome count for this species may therefore be said to be nine and ten.

Microspore tetrads show some variation in spore arrangement. Generally and typically they are tetrahedral, but other types of groupings are sometimes found, especially that often seen in monocotyledons in which the four microspores are located in the same plane (fig. 5).

On separating, the mature microspores enlarge and undergo a vacuolation, the nucleus taking a position near the wall. Here a division occurs that forms a 2-celled male gametophyte. In some instances at least, one of the daughter nuclei takes a position close to the microspore wall, where it may become separated from the sister nucleus and the main portion of the microspore contents by a wall

(fig. 6). It is not always possible, however, to observe the wall; but whether it is organized as a cell or not, the nucleus generally remains more or less fixed in position and fails to undergo further division. The nucleus in the main cavity of the microspore now divides to form the generative and tube nuclei (fig. 7). Figures 6 and 7 are stages in the development of a microspore in which a definite cell is cut off to take a position next to the wall, as just described. The division of the generative nucleus to form the sperm nuclei is shown in figure 8, while in figure 9 a mature 4-celled pollen grain is represented. In these last two stages the vacuoles seen in earlier stages have disappeared. The cytoplasm in the mature grain is rather dense and the non-functional cell by the wall is in a state of dissolution. Many mature pollen grains show but three nuclei, failure to exhibit the presence of the fourth being due to one of three causes: it may not have been formed in the first place; it may have undergone complete disintegration; or it may not have been lying in the optical section, and hence have escaped discovery. It is not known therefore in what percentage of microspores a 4-celled gametophyte arises.

To all appearances one of the daughter cells of the first division of the microspore nucleus is prothallial in nature, recalling in time of appearance and general position the first of the two prothallial cells developed in the male gametophyte history of some of the gymnosperms. Instances that occur among angiosperms, however, have been reported by CHAMBERLAIN (2) in *Lilium tigrinum;* by SMITH (9) in *Eichornia crassipes;* by CAMPBELL (1) in *Sparganium simplex;* and by WÓYCICKI (11) in *Yucca recurva*. COULTER and CHAMBERLAIN (3) regard the appearance of the fourth nucleus as sufficiently rare to cause them to hesitate in assigning to it a definite prothallial morphology. SCHNARF (8) thinks that all types of instances of excess nuclei in the male gametophyte of angiosperms may be due to such influences as light, temperature, or moisture, but particularly as the result of hybridization. Hybridization is often indicated by irregularities in meiosis and in pollen development, such as lagging chromosomes, extrusion of chromatin, polyspory, defective pollen, etc. The production of defective pollen is well marked in *A. hymenelytra*. Grains may undergo degeneration, in which instance the cytoplasm is generally intact, but fragmentation of the nuclei takes place

(fig. 10). In other instances, anthers can be found with normal pollen intermixed with empty or undersized grains, the defective portion amounting to as much as 32 per cent. In still other instances entire anthers abort and develop no normal pollen. Thus it is possible that hybridization has occurred at some time, and if so, it might be a factor in accounting for the irregularity of excess number in the male gametophyte, and also for the production of so much defective pollen. With respect to the fourth nucleus or problematical prothallial cell on the other hand, it should not be considered so remotely improbable that an angiosperm would now and then be found exhibiting an atavistic tendency sufficient to conserve an ancestral vestige such as a prothallial cell in the male gametophyte.

At the time of anthesis, the gynoecium in the staminate flower is seen to be reduced to a slender column of tissue showing no differentiation that would in any way indicate its probable morphology (fig. 11).

The embryo sac in *A. hymenelytra* is an 8-celled gametophyte.

Summary

1. The development of the pollen mother cells from the archesporium follows the usual course found in angiosperms.

2. The tapetum is composed of binucleate, richly protoplasmic cells.

3. Meiosis appears to be normal.

4. Eight autosome bivalents and a tripartite X-Y group appear on the metaphase plate in I. The X element is composed of two separate chromosomes. The N count is nine and ten.

5. Microspore tetrads show variation in grouping, the predominant type being tetrahedral.

6. Certain microspores undergo three nuclear divisions, one of the first two nuclei formed organizing as a cell that strongly resembles a prothallial cell.

7. A considerable number of pollen grains show abnormalities such as disintegrating nuclei, under size, or absence of cell contents.

8. The gynoecium in staminate flowers is reduced to a column of undifferentiated tissue.

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MARINE PLANKTON DIATOMS OF LOWER CALIFORNIA IN 1931

W. E. Allen

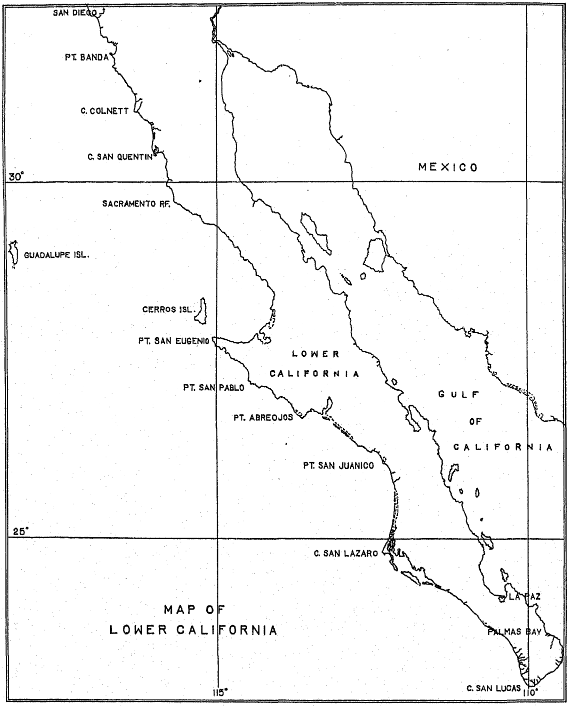
(WITH ONE FIGURE)

Introduction

At the invitation of the Commander of one of the Mexican Government patrol boats, operating in the waters off Lower California, the Scripps Institution of Oceanography assigned to Mr. P. S. Barnhart, Curator of its museum, the task of making a series of observations on fisheries conditions and occurrence of phytoplankton (ocean pasturage) in the spring of 1931. In the course of this cruise Mr. Barnhart was able to make 140 collections of surface material at numerous points along or near the west coast of Lower California and at a few points in the Gulf of California (fig. 1). All of these collections were made merely by dipping up from the surface a certain quantity of water (three gallons in this series) and filtering through a small conical net of no. 25 mill silk, with meshes averaging about 0.05 mm. in diameter.

The first catches were made on April 11, 1931, and the last on May 2, 1931, the whole series being taken in a period of about three weeks. Mr. Barnhart had made a similar cruise in 1922, on which he accumulated a series of collections of similar character and extent, but that cruise was made in July (2). A shorter series of catches obtained by the California Academy of Sciences along the west coast of Lower California in 1921 was taken in April, although a little earlier (1). While these differences in time, localities, and numbers of catches in the three series may not appear significant, it is evident from experience with occurrence of plankton organisms that they are great enough to prevent any possibility of exact comparisons. However, there is considerable information to be obtained from general comparisons.

The numbers of dinoflagellates were negligible throughout all of the catches of this series. For that reason they will receive no partic-



SAN DIEGO
PT. BANDA
C. COLNETT
C. SAN QUENTIN
30°
MEXICO
SACRAMENTO RF.
GUADALUPE ISL.
CERROS ISL.
PT. SAN EUGENIO
LOWER
CALIFORNIA
PT. SAN PABLO
GULF
OF
CALIFORNIA
PT. ABREOJOS
PT. SAN JUANICO
25°
C. SAN LAZARO
LA PAZ
MAP OF
LOWER CALIFORNIA
PALMAS BAY
115°
C. SAN LUCAS
110°

FIG. 1

ular attention. Diatoms were the only other constituents of the phytoplankton which appeared in notable numbers at any time. On that account, the remainder of this report will deal with these organisms. Records of good and bad condition, as well as of numbers present, were made for each catch.

Differences in abundance of diatoms in collections made in different localities represented in this series occurred in such a way as to make it convenient for discussion to designate four regions in the territory covered (fig. 1), only one of which gave notable evidence of productivity: (1) northern region, comprising the territory north of Pt. San Eugenio; (2) middle region, comprising the territory from Pt. San Eugenio to and including Magdalena Bay; (3) southern region, from Magdalena Bay environs to Socorro Island (not shown on this map) about 260 miles south of Cape San Lucas; (4) Gulf region, comprising the localities in the Gulf between La Paz and Cape San Lucas.

Observations

1. Northern region

On the south-bound part of the trip on April 14, 21 catches were made between San Diego and Guadalupe Island. These were taken rather near shore to Cape Banda, getting farther and farther to seaward after passing that point until about 150 miles off-shore at the island. The abundance was very low in all of these catches, although several showed the presence of a number of different species, the largest showing being nine species about 40 miles seaward from Cape Colnett. None of the five catches between Guadalupe Island and Pt. San Eugenio was as prominent as this. On the return trip a catch near Cerros Island on April 30 showed ten species, another near Sacramento Reef on May 2 showed eleven species, and another near Cape Colnett on May 2 showed thirteen species. For the region in general, such records indicate the possibility of considerable or high abundance occurring at other times, at other points not sampled, or at levels beneath the surface. It is not probable that a desert region would show this diversity of species, many representatives of which were in good condition. As a matter of fact, the Barnhart series of 1922 gave direct evidence that the indication is stronger than that of mere possibility. In that July series 23 catches out of

88 yielded numbers of diatoms greater than 10,000 cells to the liter; and in the Pt. San Quentin section 14 catches out of 39 yielded more than 10,000 and one was greater than 100,000 cells per liter, many of the smaller catches being off-shore toward Guadalupe Island.

2. Middle region

In 1931 the middle region was distinct from the others by reason of the great abundance of diatoms shown in 19 out of the 29 catches made while running southward. In fact, 13 of these catches were of the order of magnitude of millions of cells per liter, an abundance great enough to be notable in any region yet investigated by the Scripps Institution from the coast of Peru to the Aleutian Islands. For a period of three hours, approaching Pt. Abreojos on April 16, the boat ran through water of a rusty tinge ("red water" to some observers) in which the abundance of diatoms was great enough to cause the discoloration. The largest single catch in the region (3,-933,921 cells per liter) was made a little farther north near Thurloe Head (southeast of Pt. San Eugenio) at 7:15 A.M. on the same day. On the return trip on April 30 a catch yielding more than 1,000,000 cells per liter was taken in Magdalena Bay near Man o' War Cove and a fairly large catch near Cape San Lazaro, but catches following these were small, possibly in part because of being taken a little farther off-shore. In the series of 1921 and of 1922, the part of this region near Pt. Abreojos yielded the largest catches as well as the greatest number of larger catches, but in both series the largest catches were small or insignificant compared with the large catches of 1931. Still the showing of maximum abundance in the region in different years and seasons is an important indication of a tendency to good productivity in it as compared with others. Water temperatures with catches reaching more than 1,000,000 cells per liter ranged from 16.3° to 18° C.

3. Southern region

Up to and including 1931, no series of collections of phytoplankton has been obtained which showed catches of any significance from the open ocean between the general locality of Magdalena Bay and the Revillagigedo Islands nearly 300 miles to the south. In 1931 the

several catches taken in the region showed the presence of a few diatoms in every case, and it seems possible that notable abundance may occur at times not yet noticed, or that there may be notable abundance at depths below the surface which have not been sampled. It seems fair, however, to say that good productivity of plankton diatoms is not to be expected in the region.

4. Gulf region

The 1921 series is the only one which comprised collections from the Gulf of California prior to 1931. The evidence from the catches of both years agrees in showing only very small catches in the lower part of the Gulf, all being near the shores of the peninsula on the west side of the Gulf. Nine species of diatoms and seven species of dinoflagellates were represented in one of the catches of 1931, however, and it may be supposed that there are times in some years when a considerable abundance might be found.

Forms represented

In study of the material, genera of diatoms to the number of 29 were recorded, and species to the number of 46. It is probable that a number of species were overlooked, or not recognized under the low magnification used in enumeration, and a few genera may have been overlooked also; but if so their numbers must have been too small to be significant in a study mainly concerned with problems of productivity or occurrence of abundance.

Six genera, *Bacteriastrum*, *Chaetoceros*, *Coscinodiscus*, *Nitzschia*, *Rhizosolenia*, and *Thalassiothrix*, were represented in all four regions. It is probable that these genera are the six most regularly represented in the thousands of collections made by the Institution in California waters in the last 16 years. Their wide spread in the catches of this series indicates a tendency for the Lower California territory to support populations of pelagic plants generally similar in character to those somewhat farther north. In a few of the large catches of the middle region, two other genera, *Asterionella* and *Skeletonema*, contributed largely to the abundance. These two genera occasionally take the lead in abundance along the California coast, and even farther north.

Four genera and six species were especially prominent in the large catches of the middle region. Of these, *Skeletonema costatum* Grev. was most prominent in more catches than any other single species, although the catchall group, *Chaetoceros* sp., consisting of specimens too small or too poorly marked to be identified, had about equal prominence. *Chaetoceros costatus* Pav. appeared to reach the maximum numbers for a single species in a single catch (about 2,000,000 cells per liter 22 miles south of Pt. Abreojos), but its characters were often so poorly marked that the record is somewhat doubtful. *Chaetoceros socialis* Lauder presented similar aspects and difficulties, the latter increased so far as identification is concerned by its wide range of differences in the size and form of cells and in the extent of their attachment in colonies. *Nitzschia seriata* Cl. showed greatest prominence amongst the components of only one catch (11 miles south of Pt. Abreojos), and *Asterionella japonica* Cl. was first only in the catch from inside Magdalena Bay. Thirty-eight of the other species listed more or less frequently, and in larger or smaller numbers in the catches of this series, are commonly found in series of collections made in California waters and northward. Only one species, *Guinardia flaccida* (Castr.), is so rarely found in more northerly catches as to make its presence notable in Lower California. It was not abundant in any catch of this series.

Vitality of specimens

As in examinations of other series of phytoplankton in recent years, an effort was made to estimate roughly the biological condition of populations by keeping a count of those in good and those in poor or decadent condition. South-bound in region 1 nearly all specimens in the small catches were disintegrating or obviously dead. North-bound, a number of catches consisted almost entirely of specimens appearing to be in vigorous condition, judging mainly by the normal form and position of the chromoplasts. In regions 3 and 4 very few normal appearing specimens were found, except for four catches near the lower end of the Gulf of California in which most specimens were in good condition.

In the large catches of region 2 some showed nearly all in poor condition, others nearly all in good condition, but most were char-

acterized by the appearance of certain prominent species being mostly in good condition while others in the same catch were mostly in poor condition. However, *Chaetoceros socialis* was mostly in poor condition in the catches where it was prominent, while *Nitzschia seriata* was mostly in good condition where it was found in abundance. A few species of *Chaetoceros* were also mostly in good condition. These differences in showing in the same catches have two points of interest: (1) The presence of dead or decadent specimens in the surface level in such large numbers makes it difficult to explain the processes involved in flotation or deposit of such microscopic particles in nature. (2) The concurrent occurrence of abundance of species so unlike in their momentary showing of vitality is puzzling, even if it be supposed that one is replacing the other, either through superior vigor or through closer fitness. Considering the opportunity for disappearance of dead frustules by sinking, it might be expected that they would be few where vigorous development of associated forms was occurring.

Subtropical productivity

From time to time one sees some statement or account of marvelous productivity of plankton in frigid seas, sometimes coupled with a comparison with tropic seas in which negligible productivity is stated or implied. Trustworthy data covering the problem of such relative productivity in frigid and tropic seas are not in existence. For that reason, records of abundant occurrence of plankton in tropic or subtropic waters have considerable importance. In this series, catches exceeding a million cells per liter were found over a sailing distance of more than 100 miles near the border line of the tropic zone. These were all taken from the surface level which, on account of more direct sunlight, may not be the most favorable level for photosynthetic organisms, although it may be the most favorable in frigid waters where the radiation is more oblique. Even in frigid areas, it is known that the open sea, and many in-shore localities as well, show scant production at times when abundant production occurs in neighboring localities (3). Furthermore, it seems probable that marine plankton plants resemble land plants in having a longer vegetative period in the tropics than they have in frigid areas. All

things considered, it seems fair to say that while the arctic and antarctic zones cannot be proved to be most productive of marine phytoplankton by existent evidence, neither can the tropics, although such abundance at the surface level as appeared in this series gives reason for suspecting that total productivity, including all levels above 100 meters and all seasons, may be greater in the tropics or in the subtropics.

Summary

1. Great abundance of plankton diatoms was found in surface catches along the coast of Lower California from a locality near Pt. San Eugenio to a locality near Pt. San Lazaro, a distance of more than 100 miles.

2. Greatest productivity appeared near shore (relative to the open sea) and in the general neighborhood of Pt. Abreojos, the locality appearing to be most productive from the evidence of other series.

3. The diatom populations were essentially similar to those of the California coast in respect to component genera and species.

4. Certain of the larger catches contained at least as many dead or decadent specimens as there were of those in good condition.

5. The adaptability and dependability of the measured water method of collecting is well illustrated by the results of this series.

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ANOMALOUS EMBRYOS OF CULTIVATED VARIETIES OF PRUNUS WITH PARTICULAR REFERENCE TO FRUIT BREEDING[1]

H. B. TUKEY

(WITH TWENTY-TWO FIGURES)

Anomalous embryos are not infrequent among plants. Their occurrence in the species type of the sweet cherry (*Prunus avium* L.) and of the peach (*P. persica* Stokes), therefore, would be in itself of no more than passing interest. Their occurrence among cultivated clons or varieties of sweet cherry and peach, however, and particularly their frequency and character, take on added importance because of their relation to systematic fruit breeding. In addition, facts bearing on this situation are important in the commercial production of seedling understocks upon which cultivated varieties of cherry and peach are propagated. Accordingly it has seemed worth while to place on record the observations of anomalous peach and cherry embryos, made as incidental to the removal of embryos from twenty-seven varieties of peaches and seventeen varieties of sweet cherry during a study of peach and cherry development and during the artificial culturing of peach and cherry embryos (1, 2).

Some of the embryos were sectioned from material in paraffin, but most of them were studied as fresh material under the dissecting microscope. The peach varieties used were Alexander Crosby, Arp, Belle of Georgia, Canada, Carman, Champion, Chili, Delicious, Eagle Beak, Early Crawford, Early Victor, Elberta, Foster, Golden Jubilee, Greensboro, Lola, Maule Early, May Lee, Mikado, Morellone, Mountain Rose, Rochester, St. John, Schumaker, Triumph, Troth, Vainqueur, Valiant, Veteran, and Waddell. The cherry varieties used were Abundance, Black Republican, Black Tartarian, Burbank, Coe, Downer, Eagle, Early Purple, Elton, Governor Wood, Knight, Lambert, Mazzard, Napoleon, Oswego, Schmidt, and Windsor.

[1] Approved by the Director of the New York State Agricultural Experiment Station for publication as Journal Paper no. 17.

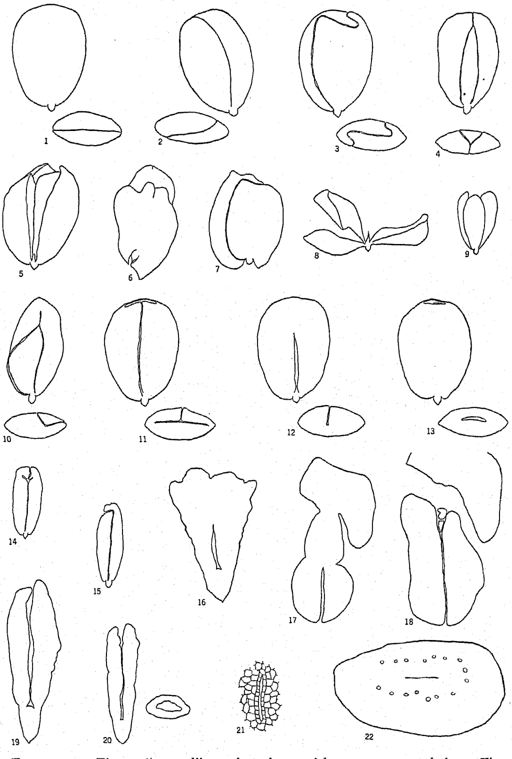
NORMAL DEVELOPMENT

The "normal" embryos in the peach and the cherry are similar, with two erect cotyledons of equal size as shown in figure 1. The seed is anatropous, so that the embryo is erect, with the hypocotyl adjacent to the micropyle and the distal end of the cotyledons adjacent to the chalaza.

ANOMALOUS DEVELOPMENT

SUPERNUMERARY COTYLEDONS.—The occurrence of embryos with more than two cotyledons has been found in five varieties of sweet cherry, Early Purple, Burbank, Lyons, Coe, and Downer, and four varieties of peach, Carman, Foster, Elberta, and Chili, from the seventeen varieties of sweet cherry and twenty-seven varieties of peach examined. The frequency is not high, however, so that the possibility is not eliminated that the examination of a greater number of embryos of the other varieties may reveal embryos with more than two cotyledons. All but one of the embryos with supernumerary cotyledons had three cotyledons (figs. 4, 7, 8). One had four cotyledons (fig. 5). Such embryos may germinate (figs. 8, 9) and some have been grown to the development of a seedling 6 inches high by artificial culture on nutrient agar (2).

SUPPRESSION OF COTYLEDONS.—The suppression of one cotyledon in whole or in part is also of frequent occurrence, although more common in the sweet cherry than in the peach. Suppression is of varying degrees. It may occur as the fuller development of one cotyledon than the other (fig. 10). It may occur as the complete suppression of one cotyledon, in which case the developing cotyledon is suggestive of the enfolding single cotyledon (scutellum) of corn (*Zea mays*), a monocotyledon (fig. 11). In other instances the cotyledons may arise undiverged to give the appearance of a closed collar (figs. 13, 16, 19, 20, 22). When the cotyledons are undiverged, the general appearance is as of a single plump cotyledon. Closer examination, however, shows a separation extending as a hollow cylinder from the tip of the cotyledons to the cotyledonary plate. Figure 21 shows in cross-section in detail the close proximity of the inner surfaces and the smallness of the opening.



FIGS. 1–22.—Fig. 1, "normal" peach embryo with two erect cotyledons. Fig. 2, cotyledons facing each other at an angle from the normal. Fig. 3, S-shaped cotyledons. Fig. 4, three cotyledons. Fig. 5, four cotyledons opened to show arrangement. Figs. 6, 7, grotesque shapes. Figs. 8, 9, germinating embryo with three functional cotyledons. Fig. 10, partial suppression of one cotyledon. Fig. 11, entire suppression of one cotyledon (note scutellum-like appearance, as of a monocotyledon). Fig. 12, entire suppression of one cotyledon. Fig. 13, lack of divergence, producing a closed collar-like structure. Fig. 14, cotyledons folded back upon themselves. Fig. 15, one cotyledon folded over the other at distal end. Fig. 16, undiverged cotyledons and proliferation of distal end. Fig. 17, cross-section of 3-cotyledon embryo at cotyledonary plate. Fig. 18, cross-section of 3-cotyledon embryo just above cotyledonary plate. Figs. 19, 20, undiverged cotyledons showing central opening from distal end to cotyledonary plate. Fig. 21, detail of central opening. Fig. 22, cross-section of cherry embryo with undiverged cotyle-

ANOMALOUS SHAPES.—It is not uncommon to find the tip of one cotyledon folded over the tip of the other (fig. 15), or to find the tips of both cotyledons folded back upon themselves (fig. 14). Still more frequently the inner surfaces of the cotyledons are not flat. Instead the vertical edges of one may practically enfold the edges of the other on one side, and the reverse on the other side, to give a vertical s-shaped appearance (fig. 3). Again, both cotyledons may be turned through nearly 90° so that they face each other at almost right angles to the normal (fig. 2). Other shapes also appear which are often grotesque, as in figures 6 and 7, and occasionally an embryo may develop several true leaves while still inclosed within the integuments.

FREQUENCY OF ANOMALOUS EMBRYOS

Anomalous embryos occur more frequently in some varieties than in others, and more frequently among horticultural varieties than among the wild species types. The Krummel peach for example may show as high as 70 per cent partially developed embryos; Eagle Beak may show as high as 20 per cent malformed embryos; and Foster may show a high proportion of abnormal embryos. On the other hand, Chili seldom shows poorly developed embryos, but frequently produces embryos with three cotyledons. The Downer cherry and representatives of the species type of *Prunus avium* (Mazzard) seldom produce abnormal embryos; likewise so-called "natural" white-fleshed peaches growing in the wild seldom produce anomalous embryos.

These observations serve to emphasize the heterozygous condition in cultivated varieties of peach and cherry, in contrast to the more homozygous condition among the wild species types. The preference of nurserymen for seed in general from wild types rather than from horticultural varieties is shown to have some basis in fact. Plant breeders might find it profitable to begin their study of segregation by an examination of the seed and the embryos from crosses. The factors of anomalous and abortive embryos are more likely to have been overlooked in *Prunus* than in other deciduous fruits, because the seeds of *Prunus* are inclosed in a stony pericarp or pit and

may therefore frequently go unobserved until the seed has either germinated or disintegrated.

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A STUDY IN THE GENUS TETRAMOLOPIUM NEES (FAMILY: COMPOSITAE)

EARL EDWARD SHERFF

Tetramolopium bennettii sp. nov.—Frutex gracilis ramosus 5–8 dm. altus, caule ramisque inferne nudis. Folia secundum ramulos ultimos subconferte disposita, plerumque suberecta, filiformia, crassa, glabrata, supra canaliculata, apice acutiuscula, 1.5–2.5 cm. longa et circ. 0.3–0.4 mm. lata. Capitula tenuiter pedunculata pedunculis minutissime glandulosis 1–3-bracteatis ramulos terminantibus tantum 1–2.5 cm. longis, circ. 1 cm. lata. Involucrum circ. 4 mm. altum, bracteis lanceolato-linearibus acerrimis plus minusve glandulosis et subciliatis. Flores ligulati 30–45, corollis circ. 3.5–4 mm. longis, limbo quam tubo vix vel non breviore. Flores tubulosi ∓16, corollis circ. 3.5 mm. longis culmine purpurascentibus pappum superantibus. Achaenia plana, oblanceolata, brunneo-straminea, adpresse erecto-hispidula, faciebus unicostata vel costa raro geminata, 2 mm. longa.

Specimens examined: *Frederick Debell Bennett* 18, Isl. Maui, Hawaiian Isls., 1833–1836 (type, Herb. Berl.); *Charles N. Forbes*, ridge east of Wainee, Isl. Maui, August, 1910 (Herb. Bishop Mus.); *idem*, Lahainaluna, Isl. Maui, August, 1910 (Herb. Bishop Mus.).

Distinguished from *T. remyi* A. Gray, which it resembles in foliage, by the smaller and more numerous heads, the much shorter peduncles, the many fewer ray florets, etc.

Tetramolopium filiforme sp. nov.—Fruticosa, humilis, ramosa, ramulis foliorum delapsorum vestigiis tectis aliter glabris. Folia numerosissima, filiformia, sparsim setosa, crassa, supra plus minusve canaliculata, apice rotundato-cartilaginea glaberrimaque, tantum circ. 1–1.5 cm. longa et 0.2–0.5 mm. lata. Capitula solitaria ultra folia breviter exserta pedunculis tenuissimis hispidulis 2–3 cm. longis, circ. 1 cm. lata. Involucrum campanulato-hemisphaericum, bracteis 2- vel sub-3-serialibus, anguste vel moderate linearibus, nitidis, apice acutis, tergo glabris vel glabratis, margine minutissime

ciliatis, intimis circ. 3 mm. longis extimis fere dimidio brevioribus. Flores ligulati circ. 30–40, lineares, reflexi. Flores tubulosi ∓12, purpurascentes, tubo limbo multo longiore. Achaenia nitida, brunnea, plana, oblanceolata, marginibus incrassatis glaberrima, faciebus longitudinaliter nunc valde nunc aegre 1-costata et superne sparsohispida, corpore circ. 2.2 mm. longa, disco epigyno parvo instructa; pappi setis albidis tenuissimis, minutissime antrorso-hispidulis, corpori aequalibus.

Specimens examined: *Dr. William Hillebrand*, Waianae Mts., Isl. Oahu, Hawaiian Isls., 1869 (type, Herb. Gray: cotypes, Herb. Berl.; Herb. Kew).

Tetramolopium polyphyllum sp. nov.—Fruticosa, forsitan humilis decumbensque, ramulis tenuibus inferne foliorum delapsorum vestigiis tectis aliter glabris. Folia caulis apicem versus confertissima (∓25 per 1 cm. caulis), demissa vel summa erecta, tenero-membranacea, angustissime linearia, apice acuta, inferne in petiolos nematoideos 1–1.5 cm. longos elongato-angustata, petiolo adjecto 2–4 cm. longa et 0.4–2.5 mm. lata, 1-nervia nervo supra impresso infra elevato, plana vel plus minusve revoluta, marginibus et nervo mediano hispido-ciliata alibi scabrido-hispidula vel mox glabrata, integerrima vel supra medium minute plus minusve serrulata serraturis in cilia brevia rigida curvato-erecta saepe desinentibus vel raro acerrime 1-vel 2-dentata dentibus inflexis usque ad 2.5 mm. longis. Capitula solitaria, exserta, tenuissime pedunculata pedunculis hispidis 1.5–3 cm. longis bracteatis bracteis filiformibus usque ad 1 cm. longis, circ. 9 mm. lata. Involucrum hemisphaericum vel late obconicum bracteis sub-3-seriatis linearibus acutis minute plus minusve hispidulis ciliatisque, interioribus marginaliter scariosis, extimis ∓1.5 mm. aliis usque ad 3.5 mm. longis. Flores ligulati ∓30, ligula lineares, revoluti, apice subtruncati subintegrique. Flores tubulosi ∓24, corollarum lobis pappum paululum superantibus. Achaenia nitida, brunnea, oblanceolata vel anguste obovata, plana, marginibus crassiuscula, faciebus plerumque unicostata sed interdum costa mediana aegra vel etiam deficiente, glaberrima vel sparsissime longisetosa setis brunneis et valde adpressis, disco epigyno parvo onusta, corpore circ. 2 mm. longa; pappo albo vel sordido-albo, setis tenuissimis minutissime antrorso-hispidulis circ. 2.5–3 mm. longis.

Specimens examined: *Dr. William Hillebrand*, Waianae Mts., Isl. Oahu, Hawaiian Isls. (Herb. Berl.); *idem*, southeast slope of Mt. Kaala, Makaka, Waianae Range, Isl. Oahu, August, 1869 (Herb. Berl.; Herb. Bishop Mus.); *Dr. Heinrich Wawra* (*Voy. Donau*) no. 2290, Isl. Oahu, 1868–1871 (type, Herb. Mus. Vienna).

Tetramolopium rockii sp. nov.—Frutex humilis, erectus vel plus minusve depressus, 3–4.5 dm. altus, ramosissimus ramulis hispidulis supra confertissime foliosis. Folia spathulata, plana, crassiuscula, apice obtusa vel orbiculata, inferne sensim in petiolum validum rigidum subplanum marginatum hispido-ciliatum 4–8 mm. longum angustata, obsolete 1-nervia, faciebus rugoso-papillatis plus minusve hispidula glandulosaque, margine hispido-ciliata et integra vel apicem versus minutissime serratulata, 1–1.8 (rarius –2.7) cm. longa et 2–3.5 (rarius –6) mm. lata. Capitula solitaria circ. 1.2–1.5 cm. lata, pedunculis subvalidis hispidulis paucibracteatis ramulos terminantibus 5–12 cm. longis. Involucrum 5–6 mm. altum, bracteis numerosis oblongo-linearibus acutis extus glandulo-hispidulis. Flores ligulati 60–80, albidi, ligula tubo paulo breviore. Flores tubulosi numerosi, sicci subalbidi, corollarum lobis exsertis. Achaenia stramineo-fulva, obovata, plana, faciebus antrorsum adpresso-hispidula et perspicue uninervia, marginibus calloso-incrassata et aegre erecto-hispida, corpore circ. 2 mm. longa, pappo albo circ. 3 mm. longo.

Specimens examined: *Joseph F. Rock* 10299, on beach, Moomomi, Isl. Molokai, Hawaiian Isls., March, 1910 (type, Herb. Gray: cotypes, Herb. Berl.; Herb. Bishop Mus.; Herb. Field Mus.).

Tetramolopium lepidotum (Less.) comb. nov.; *Erigeron lepidotus* Less., Linnaea **6**:502. 1831; *Vittadinia chamissonis* A. Gray, Proc. Amer. Acad. **5**:119. 1861; *Tetramolopium chamissonis* Hillebr. Fl. Haw. Isls. 199. 1888.—The type material of LESSING's species had been collected by *Von Chamisso* on the Island of Oahu, 1816–1817. LESSING mentioned having seen several of the dried specimens. The Berlin sheet is from KUNTH's private herbarium and bears three specimens given to KUNTH by *Von Chamisso* in 1831. While any *Von Chamisso* material which might still be extant at Halle would by some be taken as the type, the Berlin sheet is thoroughly ample for all practical purposes. One specimen is the form described by LESSING for his *Erigeron lepidotus*, having "folia . . . . . . 6–9'''

longa," and later described by ASA GRAY as *Vittadinia chamissonis.* The other two specimens are larger and have leaves 3–5.5 cm. long. These match the type material at Kew of *Erigeron pauciflorus* Hook. & Arn. and the type material in Berlin of *Tetramolopium chamissonis* var. *luxurians* Hillebr., and are to be distinguished varietally as:

TETRAMOLOPIUM LEPIDOTUM var. **luxurians** (Hillebr.) comb. nov.; *Erigeron pauciflorus* Hook. & Arn. Bot. Beechey's Voy. 87. 1832; *Tetramolopium chamissonis* var. *luxurians* Hillebr. Fl. Haw. Isls. 199. 1888.

TETRAMOLOPIUM HUMILE **sublaeve** var. nov.—A specie plurimis foliis sparsim et saepe irregulariter setosis vel etiam setis omnibus deficientibus glabratis sed minutissime glandulo-punctatis, pedunculis 3–6-congregatis in unico ramo differt.

Specimens examined: *The United States South Pacific Exploring Expedition under Captain Wilkes*, Isl. Hawaii, Hawaiian Isls., 1840 (type, Herb. U.S. Nat.: cotype, Herb. Gray).

ASA GRAY at first had recognized this in the herbarium as a variety but later included it as a variation in his original description of the species proper ("Variat foliis hirsutioribus vel subglabratis"). The habit of having several peduncles on each branch is, however, quite distinctive and may be relied upon to separate this variety, as also the next following variety, from the species itself.

TETRAMOLOPIUM HUMILE **skottsbergii** var. prim. nomin.; *Tetramolopium humile* var. *β*, Hillebr. Fl. Haw. Isls. 199. 1888; *Tetramolopium humile* f. *laxum* Skottsb., Meddel. Göteborgs Bot. Trädgård **2**:273. 1926 (*ex num. sed sine descript.*).—Planta plus erecta et saepe minus hispida. Pedunculi plerumque 3–6-congregati in unico ramo et breviores, capitulis minoribus, floribus paucioribus. Achaeniorum facies obscure uninerviae praecipue ad basim.

Specimens examined: *Charles N. Forbes* 848*H*, Humuula Trail to summit of Mauna Kea, Isl. Hawaii, Hawaiian Isls., June 14, 1915 (Herb. Bishop Mus.); *Dr. William Hillebrand*, Central Plateau, Isl. Hawaii (type, Herb. Berl.); *Albert S. Hitchcock* 14278, alt. about 11000 ft., loose lava, slope of one of the peaks, Mauna Kea, Aug. 22, 1916 (Herb. Bishop Mus.; Herb. U.S. Nat.); *Jules Remy* 244, Isl. Hawaii, 1851–1855 (Herb. Gray); *Joseph F. Rock* 8333, alt. 10000 ft., Mauna Kea, June, 1910 (Herb. Bishop Mus.; Herb. Field Mus.;

Herb. Gray); *Carl Skottsberg* 703, alt. 3000 m., Mauna Kea, Sept. 28, 1922 (Herb. Goth.); *United States South Pacific Exploring Expedition under Captain Wilkes*, Isl. Hawaii, 1840 (Herb. Gray; Herb. U.S. Nat.; *cum specie ipsa commixtum*).

Named in honor of DR. CARL SKOTTSBERG, Director of the Arboretum of Gothenburg, who had given particular attention to this form, even designating it (*loc. cit.*) f. *laxum*, but without description.

TETRAMOLOPIUM ARENARIUM **confertum** var. nov.—Ramosior ramulis plerumque sub 1 dm. longis. Folia 1.5–3 cm. longa et 3–5 mm. lata apicem versus utrinque saepe 1-dentata dente acri. Capitula conferta pedicellis non vel vix manifestis, demum 4–6 mm. lata, involucro 2.5–3.5 mm. alto.

Specimens examined: *Dr. William Hillebrand* and *Rev. J. M. Lydgate*, growing 2–4 ft. tall, Nohoananohea[1] near Waimea, Isl. Hawaii, December, 1872 (Herb. Bishop Mus.); *Lydgate, eodem loco et tempore* (type, Herb. Berl.).

TETRAMOLOPIUM CONYZOIDES var. **dentatum** (H. Mann) comb. nov.; *Vittadinia conyzoides* var. *dentata* H. Mann Enum. Haw. Pl. *sub num.* 203, Proc. Amer. Acad. **7**:173. 1867.

Tetramolopium arbusculum (A. Gray) comb. nov.; *Vittadinia chamissonis* var. *arbuscula* A. Gray, Proc. Amer. Acad. **5**:120. 1861; *Tetramolopium chamissonis* var. *arbusculum* Hillebr. Fl. Haw. Isls. 199. 1888.

TETRAMOLOPIUM CONSANGUINEUM **leptophyllum** var. nov.—Folia anguste linearia, utrinque saepe 1–2-denticulata dentulis digito parvo similibus $\mp$0.6 mm. longis, 3–4.5 cm. longa et 1–1.5 mm. lata.

Specimens examined: *The United States South Pacific Exploring Expedition under Captain Wilkes*, Waimea, Isl. Hawaii, Hawaiian Isls., 1840 (type, Herb. N.Y. Bot. Gard.: cotype, Herb. Gray, *cum specie ipsa commixtum*).

CHICAGO NORMAL COLLEGE

[1] Spelling taken from HILLEBRAND's label in Berlin. LYDGATE's spelling is Nohoananohae.

VEGETATIVE REPRODUCTION IN CAMPTOSORUS RHIZOPHYLLUS

ILDA McVEIGH

(WITH EIGHT FIGURES)

Introduction

Although regeneration and adventive growth are familiar phenomena in plants, the tissues concerned have been studied in relatively few species. NAYLOR (8) presents an exhaustive review of the literature on these and allied subjects, and finds that in the species in which their origin has been histologically studied, new parts arise from embryonic or slightly differentiated cells. No clear evidence of any considerable loss of differentiation was found.

Among ferns many examples are known of the adventive origin of new individuals. Both sporophytic and gametophytic tissues are produced adventitiously by fern gametophytes (5, 9, 10, 11). The new structures formed by such plants must originate from parenchymatous or from embryonic cells, since these are the only kinds present.

New plants arise from buds formed on the leaves of *Phegopteris sparsiflora*, *Acrostichum punctulatum*, *Pteridium aquilinum*, *Aspidium filix-mas*, *A. spinulosum*, *Woodwardia radicans*, *Chrysodium flagelliferum*, *Asplenium bellangeri*, *A. bulbiferum*, *A. viviparum*, *A. platyneuron*, *Aspidium aculeatum*, *Ceratopteris thalictroides*, *Woodsia obtusa*, *Cystopteris bulbifera*, and *Diplazium celtidifolium* (1, 2, 4, 6, 7). In *Pteridium aquilinum*, *Aspidium filix-mas*, and *A. spinulosum* the buds appear very early on the petioles, before development of the blades and differentiation of the tissues have begun. In *Woodwardia radicans* and *Chrysodium flagelliferum* the buds appear at the tips of the arched leaves. The buds of *Asplenium bellangeri*, *A. bulbiferum*, *A. viviparum*, *Diplazium celtidifolium*, and *Cystopteris bulbifera* have their origin in single isolated epidermal cells of the leaves, or in single superficial cells of the young leaf before the differentiation of the epidermis.

In *Platycerium*, *Asplenium esculentum*, *A. platyneuron*, *Ophioglos-*

sum vulgatum, and *Woodsia obtusa* new plants arise from buds produced on the roots (2, 7). In *Platycerium* and *Asplenium esculentum* the apex of the root may be directly transformed into a leafy bud. The buds of *Ophioglossum vulgatum* have their origin in the segments cut from the apical cell of a root.

Aposporous prothalli or prothallus-like tissues are produced by *Phegopteris polypodioides*, *Athyrium filix-foemina*, *Lastrea pseudo-mas* var. *cristata*, *Scolopendrium vulgare*, *Dicksonia punctilobula*, *Polystichum acrostichoides*, *Asplenium platyneuron*, *Woodsia obtusa*, *Cystopteris fragilis*, *Osmunda regalis*, *Pteris cretica* var. *albo-lineata*, *Aspidium marginale*, and *Woodwardia virginica* (3, 5, 7). In most of these species the prothalli are formed from detached primary or secondary leaves, but in *Asplenium platyneuron*, *Woodsia obtusa*, *Aspidium marginale*, and *Woodwardia virginica* they are formed also from detached roots. In *Woodwardia virginica* the gametophytic tissue arises from single cells on the surface of the leaves, or from cells on the surface or near the surface of roots.

The adventitious structures of ferns, so far as has been reported, have their origin in parenchyma cells, epidermal cells (usually when young), or embryonic cells. The present paper reports the results of a detailed study of the origin of new plants in *Camptosorus rhizophyllus*.

Material and methods

Coiled tips of leaves of *Camptosorus* were collected near Columbia, Missouri, killed and fixed in Carnoy's fluid, carried through the customary paraffin technique, cut 8–10 μ in thickness, and mounted serially. Most of the sections were made in a vertical plane parallel to the longitudinal axis of the leaf; a few sections were made perpendicular to the longitudinal axis. The sections were stained in Delafield's haematoxylin. Leaf tips and embryonic plants were preserved in formalin-alcohol, and the externally visible parts studied with the binocular microscope.

Observations

Structure of leaf tips

The leaves of *Camptosorus rhizophyllus* are simple, evergreen, and somewhat coriaceous. All are more or less cordate at the base but vary from obtuse to acuminate at the tip. The first leaves of a plant

are usually obtuse, but in older plants the differently shaped leaves are formed apparently in no definite order. The leaves have the usual circinate vernation. The long acuminate tips remain coiled but the obtuse and acute tips soon straighten. All types of leaves bear spores. The sori are oblong or linear and are found irregularly scattered on either side of the reticulate veins; those next to the midrib occur singly and the outer ones are usually arranged in pairs. Indusia are laterally attached.

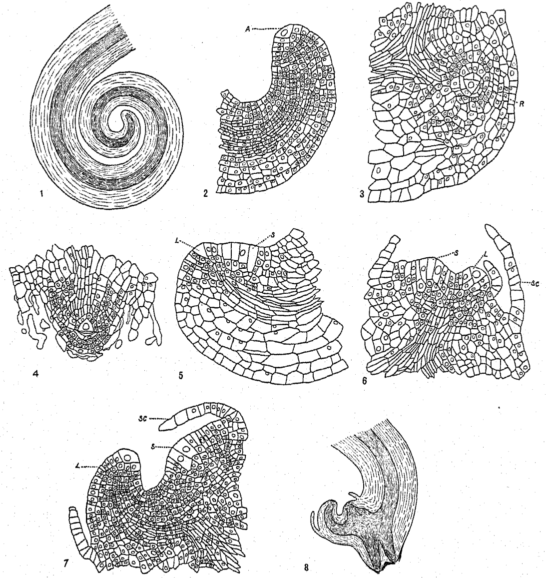
As the acuminate leaf tapers toward the tip it gradually changes in shape from a flat structure to one that is nearly cylindrical. Many glandular trichomes, each composed of three or four deeply staining cells, are found on the leaf near the tip.

Figure 1 represents diagrammatically the coiled leaf tip as seen in vertical longitudinal section. In figure 2 the extreme tip is represented in detail. The partially differentiated vascular tissue extends longitudinally through the center of the leaf almost to the tip. The cells on the inner (ventral) surface of the curve are small, their walls are thin, their nuclei are proportionally large, and they stain more deeply than the other cells of the tip. Many mitotic figures are found in this region. At the extreme tip and a little to the ventral side is a single large apical cell[1] (fig. 2). On its inner two faces are the cells which have been most recently cut off from it. The cells on the dorsal side of the leaf tip appear somewhat larger and more mature. A cross-section of the tip reveals the same difference between dorsal and ventral portions of the tip.

Formation of new plant

When the new plant is about to appear, the first change externally noticeable is in the shape of the parent leaf, which uncoils until only the extreme end is bent. This part of the leaf thickens and becomes somewhat club-shaped. Several slight protuberances are found on the surface of this structure. On the ventral surface is a relatively large rounded mass of tissue surrounded by scales. This is the apex

[1] Kupper (Flora **96**:361. 1906) has earlier discussed vegetative reproduction in *Camptosorus* (*Scolopendrium*) *rhizophyllus*. He states, "Die jungen Blätter verlieren sehr früh die Scheitelzelle und wachsen darauf mit Randzellen weiter." My investigation shows (fig. 2) that the apical cell not only persists until the leaf is mature but also (figs. 5, 6, 7) gives rise to the first leaf of the new plant. Kupper makes no statement concerning the plane in which his sections were made, and it is difficult to determine this from his figures.



FIGS. 1–8.—Fig. 1, diagram of vertical longitudinal section of leaf tip showing course of vascular strand. $\times 20$. Fig. 2, vertical longitudinal section of leaf tip before vegetative reproduction has started (*a*, apical cell). $\times 110$. Fig. 3, dorsal portion of vertical longitudinal section of leaf tip showing root primordium (*r*, apical cell of root). $\times 110$. Fig. 4, longitudinal section through young root which has just broken through dorsal cells of leaf. $\times 110$. Fig. 5, vertical longitudinal section through leaf tip in early stage in development of the first leaf and stem of the new plant (*l*, apical cell of first leaf; *s*, apical cell of stem). $\times 110$. Fig. 6, ventral portion of vertical longitudinal section of leaf tip showing later stage in development of the first leaf and stem (*s*, stem tip; *sc*, scale). $\times 110$. Fig. 7, ventral portion of vertical longitudinal section of leaf tip showing later stage in development of first leaf and stem of new plant (*l*, first leaf). $\times 110$. Fig. 8, diagram of vertical longitudinal section of leaf tip which has started to develop into a new plant, showing vascular connections between parent leaf and parts of new plant.

of a new leaf; it is derived from the continued growth of the old one. On the lateral and dorsal surfaces are slight swellings, and the cells just beneath the surface in these areas are brown and apparently dead. In a later stage finger-like projections are visible on the sides where formerly the slight swellings were observed. These are roots which have forced their way through the outer cells of the leaf and are still surrounded by some of the dead surface cells which have not sloughed off. Their number, although variable, is usually five or six; those on the lateral surfaces appear first.

When the scales surrounding the bud are removed, the embryonic leaf and stem tip are visible on the ventral surface. The stem tip appears as a slight protrusion just proximal to the first leaf, a second embryonic leaf appearing lateral to the stem tip. Later the leaves arise on all sides of this tip. Only the extreme tip of the latter, well protected by scales and leaf bases, projects above the points of attachment of the leaves. The first leaves are small and usually somewhat obtuse, although their shape varies considerably. None of the first leaves remain coiled at the tip as do the older ones. The first leaf is the smallest. Well developed plants with several leaves and roots are found still attached to the parent leaves.

Sections through tightly coiled tips do not show the beginning of the growth of new parts; this is evident, however, in sections through tips that are beginning to uncoil. Vertical longitudinal sections are best for this study because of the dorsiventral orientation of the parts of the young plant.

Roots arise first in the ontogeny of the new plant. Development starts while the leaf tip is still somewhat coiled. In a section of a single leaf tip the roots are cut at different angles; this increases the difficulty of determining their origin. They arise endogenously and grow through the outer cells of the leaf tip in much the same manner as ordinary roots emerge through the cortex of a primary root.

It is probable that the first roots originate from cells in the embryonic region of the parent leaf tip. Figure 3 illustrates an early stage in the development of a young root. This root primordium is found in the embryonic region of the leaf tip just beyond the end of the procambial strand. The apical cell is discernible early in the ontogeny of the root. Some cells between the root primordium and

the procambial strand are still embryonic. It is possible also that roots originate in the procambium before the differentiation of the vascular tissues. It may be that the root initial is laid down at the same time as the cells of the procambial strand, or that one of the cells of the procambial strand becomes the root initial. The root initial thus formed may commence growth at once and so be of like age, in its development, with the terminal part of the leaf vein.

The young roots presumably force the outer cells of the leaf tip apart as they grow to the surface. Figure 3, which shows a cross-section through a young root, shows also the disorganized condition of the outer cells of the leaf tip caused by another root tip just dorsal to the one shown in the figure. Traces of the vascular strand and some of the outer cells of this root are also visible.

A median longitudinal section through a young root that has just broken through the epidermis of the parent leaf is shown in figure 4. At this stage the root apex is protected by a well differentiated root cap. Longitudinal and cross-sections of roots show that the growing point of the root is occupied by a tetrahedral apical cell. Cells are cut off successively from the four sides; those cut off from the distal side, which is usually somewhat more curved than the others, develop into the root cap. In this respect the roots resemble those formed normally from the stem of a fern.

Figure 5, a vertical longitudinal section through the tip, illustrates the origin of the first leaf. Instead of the single large apical cell which ordinarily occupies the tip of the leaf (fig. 2), there is a slight swelling, composed of embryonic cells, terminating in a small apical cell. This apical cell has the same shape as that of the parent leaf and occupies approximately the same position. The rate of cell division probably increases at this time, since the apical cell and the other cells of the new leaf surrounding it are smaller than those in the corresponding part of the tightly coiled tip. The first leaf of the new plant evidently originates from the apical cell of the parent leaf.

A later stage in the development of the leaf is shown in figure 6. The protrusion shown in figure 5 has increased somewhat in size. The apical cell is much larger in proportion to the surrounding cells than in the preceding figure, indicating that the increase in cell number is due more to the division of those cells formed by the apical

cell than to the division of the apical cell itself. The young leaf apex is protected by scales, which converge over it. These scales arise from the base of the bud.

Still another stage in the development of the leaf is shown in figure 7. The base of the leaf is thicker than the tip. Because the dorsal side enlarges more rapidly, the apical cell is forced to the ventral surface, and the tip becomes coiled.

The stem tip is located on the ventral surface of the parent leaf tip, proximal to the embryonic leaf (fig. 5). It appears first as a small protrusion separated from the embryonic leaf by a slight indentation in the surface. The cells in this indented portion are very small, while those on either side are actively dividing and enlarging. This is better illustrated in figures 6 and 7. The apex of the stem is occupied by a large initial cell which is much deeper than it is broad. In shape this cell resembles the segments cut off from the apical cell of the parent leaf. It is probable, therefore, that the stem originates from a segment of the apical cell of the parent leaf. In sections of older plants the stem tip does not protrude any more than in the figures just described. The roots, stem, and leaf and their relative positions are illustrated diagrammatically in figure 8. The vascular connections which soon arise between the new parts and the vascular bundle of the parent leaf are also illustrated.

The young plant formed by vegetative reproduction at the tip of the leaf is very similar (in, for instance, the stage illustrated in figure 6) to the embryo which results from syngamy. The shape of the first leaf and of the stem and their position with regard to each other are the same in both. The leaf apical cells of both are alike. The stem apical cells are alike, but differ from those of the leaves. The young plant which results from vegetative reproduction possesses several roots formed approximately at the same time and formed in the way later roots are formed on a plant, while the embryo has at first only one root. The roots of the two young plants resemble one another in structure, each having a single tetrahedral apical cell protected by a root cap. In the young plant produced vegetatively, nothing corresponding to a foot was observed. In this respect, it resembles embryos of apogamous origin (10).

Summary

1. The new plants formed by *Camptosorus rhizophyllus* arise in the embryonic region of the leaf tip.

2. The first leaf of the new plant is formed by the continued growth of the apical cell of the parent leaf.

3. The stem arises from cells in the embryonic part of the leaf tip, probably from one of the segments of the apical cell of the parent leaf.

4. The roots originate probably in two ways, from cells in the embryonic region of the leaf tip and from cells of the procambial strand.

5. In general, the condition in *Camptosorus rhizophyllus* is similar to that found in the majority of other species histologically studied, that is, adventitious structures have their origin in either undifferentiated or only slightly differentiated cells.

I wish to express appreciation to Dr. H. W. Rickett and to Dr. E. E. Naylor for helpful advice and criticism during this investigation and in the preparation of this paper. Acknowledgment is due Miss Coral Fleenor for help with the illustrations.

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PHYSICAL LAWS AND THE CELLULAR ORGANIZATION OF PLANTS

R. B. THOMSON AND KATHLEEN L. HULL

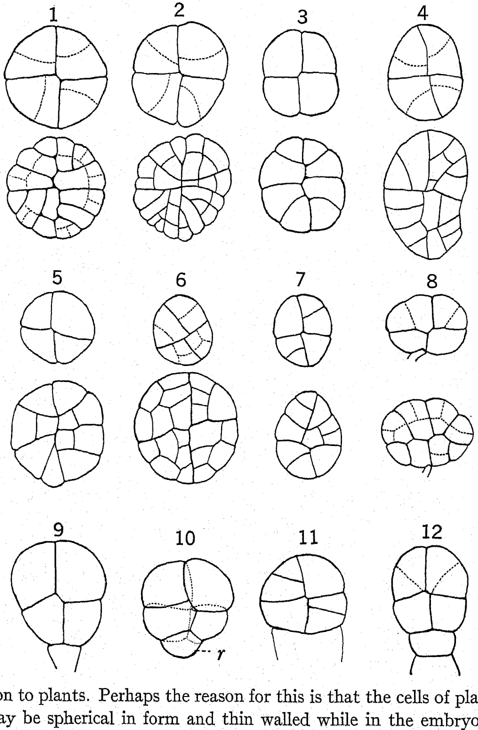
(WITH TWELVE FIGURES)

Since 1897, when ROUX (5) performed the experiments by which he demonstrated the essential similarity between a suitable system of oil drops and the early segmental stages of the egg of certain animals, there has been a sporadic but slowly growing interest in this field of research. The work of THOMPSON (8) has been perhaps the most stimulating. He demonstrated mathematically how the laws of minimum surface were applicable to stability of form. Two figures illustrating his theoretical requirements have been reproduced here (fig. 1). THOMPSON showed that the greatest stability in the quadrant aggregate was attainable when the members were asymmetrically arranged and there was a "dorsal furrow" (center of figure). At the third division a particular angle of anticlinal wall fulfilled the theoretical requirement (fig. 1, dotted line). When periclinal walls appeared in later stages these were accompanied by the more stable anticlinal type. The close correspondence between the theoretical requirements and actual organization was illustrated by THOMPSON in *Erythrotrichia* (fig. 2).

The applicability of these laws to the embryogeny of other animals was soon recognized, but it seems not to have been considered that the organizing tissues of plants might conform to such laws. This is possibly because a very thin and elastic wall is necessary for the fulfilment of the theoretical requirements, and a thick and firm wall is characteristically present in mature plant cells. Possibly also the widespread phenomena of filamentous growth in plants have been a deterrent factor, since the laws are applicable only to cells which have isodiametric symmetry, that is, are of spherical form.

Recently our attention was drawn to the possibility of the application of these laws to plants. In making a comparative study of embryogeny such uniformity was found in the cellular organization of the young embryo from bryophytes to seed plants that we were led to suspect that there was some fundamental factor involved. When

the forms of the cells were compared with the theoretical requirements, it was concluded that if the laws of minimum surface were applicable to the development of animals, they could also find applica-



tion to plants. Perhaps the reason for this is that the cells of plants may be spherical in form and thin walled while in the embryonic condition, and so can conform to the requirements as well as can those of animal cells.

After reaching the conclusion that the laws might apply to plants, many illustrations were found in the literature as well as in our own preparations. The tissues involved were from a variety of embryonic sporophytes and developing gametophytes, and indicated a wider applicability of these laws than had at first been suspected. This was made more evident by finding from our own material that drawings made in illustration of articles may be inaccurate and not represent the actual conditions. For example, the dorsal furrow may be omitted and the four cells represented as symmetrical. Often too the angle of anticlinal walls has not been properly drawn.

In order the better to direct attention to the applicability of these laws to plants, we have included outline drawings of the early cellular organization of a variety of forms. The figures are practically self-explanatory and only brief comments are needed. Figure 3 shows the organization of the teliospores of *Uredinopsis osmundae* drawn from a preparation made by Mr. S. M. Pady. In figure 4, stages of the sporophyte of *Targionia hypophylla* are illustrated (from Campbell 2). The drawings (fig. 5) of the young embryo of *Osmunda cinnamomea* are from our own preparations. Figure 6 illustrates the monocotyledon *Pistia*, while figure 7 shows the condition in a dicotyledon, *Acacia*, the former from Hegelmaier (4) and the latter from Guignard (3). In figure 8 the young gametophyte of *Angiopteris evecta* is illustrated from the recent work of Schmelzeisen (6). In this case the upper cells have developed in advance of the lower, owing, Schmelzeisen considers, to the differential influence of light. A rhizoidal outgrowth too has begun to develop from one of the lower cells.

Figures 1–8 illustrate masses of cells which have come immediately from the development of a single cell, either gametophytic or sporophytic. In many plants the spore or fertilized egg cell does not develop immediately into a solid mass, there being an intervening stage which is often filamentous. In this case the delayed massive stage may also show conformity with the theoretical requirements. Figures 9 and 10 illustrate this condition in two gametophytes (*Osmunda* and *Equisetum*, redrawn from Campbell 2). Figure 11 is of a proembryo of *Pinus* (after Buchholz 1), and figure 12 of *Urtica* (after Souèges 7).

Although we hold no brief for the applicability of physical laws to either plant or animal organization, it is felt that there are possibilities in the application of these laws to plants which should not be overlooked. For example, in work on embryogeny we have found that the literature, especially that of vascular cryptogams, is pervaded by the idea that the initials of adult organs appear very early. Some investigators label even the quadrant cells, stem, leaf, root, and foot. Others see in certain suggestively shaped cells of the primary massive stage the definitive initials of the adult organs. This has led to much confusion over the allocation of different organ initials to the various segments of the proembryo, and many of the contradictory statements in the literature can undoubtedly be traced to the mistaking of these numerous spurious initials for the true ones.

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* We have not been able to consult ROUX's original work, but it and other literature dealing with the subject have been critically reviewed in JAMES GRAY's Textbook of experimental cytology (Cambridge University Press. 1931).

BRIEFER ARTICLES

STANDARDIZATION OF METHOD FOR DRAWING ALGAE FOR PUBLICATION

(WITH NINETEEN FIGURES)

Up to the present time no standard system of magnification in drawing algae for published articles has been adopted. A plan used in the phycological laboratory of the University of Minnesota is briefly described in this note.

Since most botanical journals and texts have pages measuring approximately 5×7 inches, a standard size of 4×6.2 inches has been adopted. This leaves space at the bottom of the illustration for the legend or explanation of the figures.

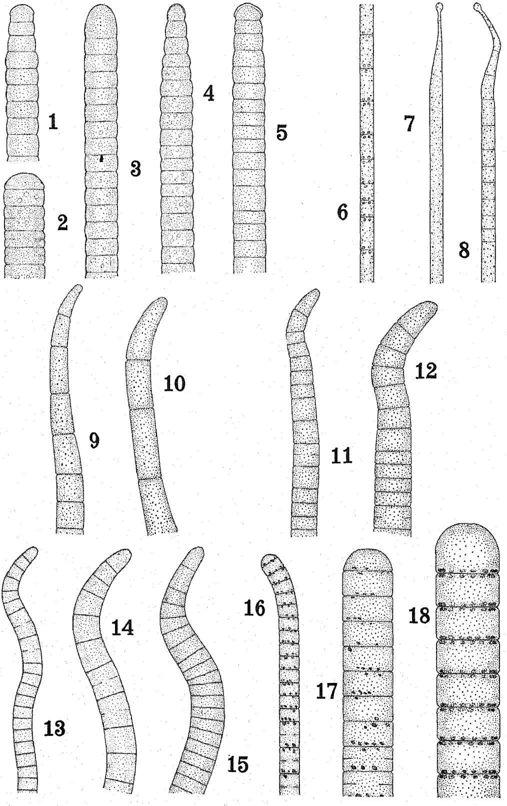
TABLE I

Direction to artist		Direction to photographer	
Diameter of cell (μ)	Amount of magnification	Size of sheet (inches)	Size of print (inches)
1.0–3.5	$1\ \mu=10$ mm.	20×31	2×3.1
4.0–9.5	$1\ \mu=5$ mm.	20×31	4×6.2
10.0–24.5	$1\ \mu=2$ mm.	8×12.4	4×6.2
25.0–75.0	$1\ \mu=1$ mm.	4×6.2	4×6.2

A micrometer eyepiece is necessary. The value of the scale divisions must be ascertained for the high power objective and eyepiece combination to be used. An oil immersion objective should be provided for objects less than $5\ \mu$ in diameter.

The first step in the process is to obtain the diameter in micra of one of the typical cells in the plant to be drawn. On the basis of this measurement a suitable factor for magnification of the object in the reproduction may be determined. The factors employed in this laboratory are given in table I.

In making the drawing, each true measurement must be multiplied by the proper factor. A cell measuring $3\times 5\ \mu$ would be drawn 30×50 mm. in size. In such a drawing all details may be properly brought out. Curved



FIGS. 1–18.—Species of *Oscillatoria:* figs. 1–5, *O. rubescens;* figs. 6–8, *O. splendida;* figs. 9, 10, *O. cortiana;* figs. 11, 12, *O. okeni;* figs. 13–15, *O. terebriformis;* figs. 16–18, *O. tenuis.* ×1000. (Drawn by MARJORIE A. FORBES.)

19

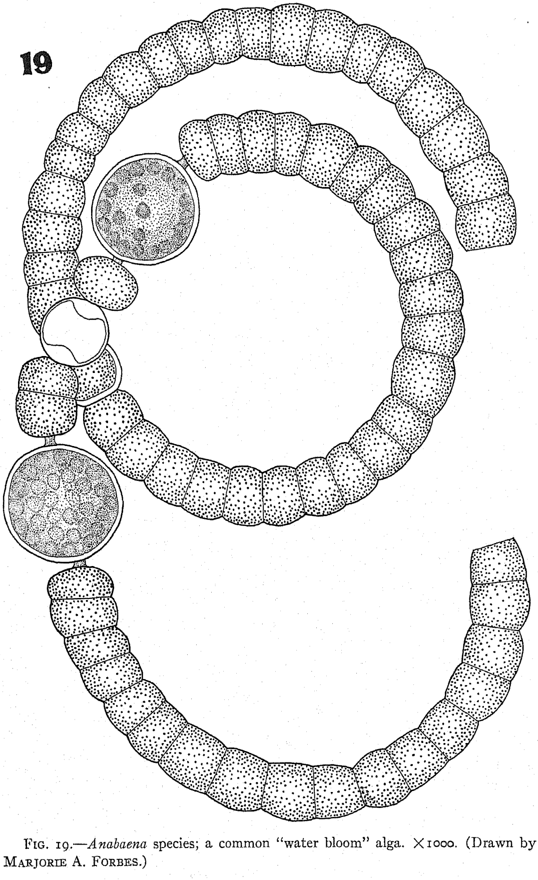


FIG. 19.—*Anabaena* species; a common "water bloom" alga. ×1000. (Drawn by MARJORIE A. FORBES.)

lines and tapered or hooked end cells may be exactly depicted. Finally, before being traced on a good grade of paper, the drawing must be checked by measuring longer distances between any two points here and there, as from the apex of the trichome to the base of the tenth cell, for example. A cell measuring 8 μ in diameter should be drawn 40 mm. wide; one measuring 20 μ should be drawn 40 mm. wide; while if the cell is between 25 and 75 μ in diameter, multiplication is unnecessary since the factor 1 is used.

The figures are now traced on drawing paper which will take India ink well. Pen points of the proper type[1] must be used for inking the drawings.

After the drawings are made they are cut out and attached, with rubber cement, to a sheet of firm white paper of the proper size, ready for the engraver. The sheets should be laid out very carefully with soft pencil lines setting off the margins. The space on the sheet inside the margin lines should measure exactly 20×31 inches, in case the factor 10 or 5 was used in drawing; 8×12.4 inches, with the factor 2; 4×6.2 inches, if the factor 1 was used. The drawings are now to be arranged as indicated by figures 1–19. They should be placed farther apart on the larger plates and closer together on the smaller ones.

In photographing the illustrations, care must be taken to have the sheets reduced exactly to certain diameters, according to the magnification used in drawing. In the objects multiplied by 10, the sheet, 20×31 inches, must be reduced to a print measuring exactly 2×3.1 inches. Four of these will make an illustration measuring 4×6.2 inches. If the object was multiplied by 5, the sheet, 20×31 inches, must be reduced to a print measuring exactly 4×6.2 inches. Likewise the sheet measuring 8×12.4 inches must be reduced to a print 4×6.2 inches in size. The result will be that, in every case, the figures on the published plates will represent a magnification of exactly 1000 diameters.—JOSEPHINE E. TILDEN, *University of Minnesota, Minneapolis, Minnesota.*

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[1] PEN POINTS TO BE USED FOR:	MAKE OF PEN POINT:
Factor 10	Esterbrook no. 788, oval point
Factor 5	Leonardt no. 516 E.F., ball point
Factor 2	Leonardt no. 516 E.F., ball point, or an old Gillotte no. 404
Factor 1	Gillotte no. 404 or Dietzgen no. 3440

CURRENT LITERATURE

Flora of the southeastern United States

In a long awaited and but recently published volume,[1] SMALL has presented a noteworthy treatment of "the species of nature and naturalized flowering plants known by the author to grow naturally in the southeastern United States south of the northern boundaries of North Carolina and Tennessee and east of the Mississippi River." These total 5557, distributed among 1518 genera and 233 families. The volume might pass for a much revised third edition of SMALL's Flora of the southeastern United States, first published in 1903; it deals, however, with a more restricted area. Within the range covered, it easily eclipses all floristic works of the past and will doubtless be the commonly accepted authority for many years to come. In a general way the sequence of plant families is that employed in ENGLER and PRANTL's Die Natürlichen Pflanzenfamilien. Conventionally enough, a logical English equivalent accompanies the scientific name of each family, such as, for example, Primrose Family for Primulaceae, and Gentian Family for Gentianaceae. For orders, however, there is used the quite different method of modifying the scientific name itself. Thus the Primulales are the Primulal Order, the Gentianales the Gentianal Order, etc. Pteridophyta are omitted. In numerous cases the descriptions have been abbreviated. A valuable feature is "a drawing showing a flower and its gross morphology and a fruit, of the first species under each genus given." The author disclaims (p. v) having been influenced in his treatments of the various groups by traditional "lumping" and modern "splitting." Generally speaking, however, the larger families and genera have been broken down into smaller groups, so that the subfamilies and generic sections of many authors are shown respectively as families and genera. Numerous sections of earlier authors have, moreover, been designated subgenera (e.g., *Eucoreopsis*, *Anacis*, *Silphidium*, and *Calliopsis*, p. 1446), thus further overloading our already heavily encumbered nomenclature without apparent gain to taxonomy. Further confusing the student, the status of subgenus is no sooner proposed than it is in various cases dropped or at least ignored and a new set of names given (e.g., Lanceolatae, Palmatae, Latifoliae, etc., p. 1446), without designated status, but intended manifestly as mutually coordinate and yet created in some cases for a subgenus and in others for a mere fractional group lower than a subgenus in rank. Too often distinctions relied upon for such appellations become wholly or in part obliterated as subsequent cosmopolitan monographs are produced. Hence, aside from the matter of needlessly dupli-

[1] SMALL, J. K., Manual of the southeastern flora. 8vo. pp. xxii+1554. Illustrated. Published by the author, New York (press of the Science Press Printing Co., Lancaster, Pennsylvania). 1933.

cating names or of forfeiting claims to consistency, it would seem that authors of manuals, dealing as these commonly do with more or less restricted ranges, should exercise the utmost reserve in the delimitation of sectional or other subgeneric groups.

Minor variations of species, "varieties" or "subspecies," are said in the preface not to have been considered to any great extent. It must be noted, however, that many of the "species" listed (e.g., *Euonymus obovatus*, p. 817; *Bidens nashii*, p. 1453; *Coreopsis crassifolia*, p. 1447; and *C. lewtonii*, p. 1449) are by various other authors construed as mere varieties. A short and all too inadequate appendix of 3 pages appears toward the end of the volume. Recording as it does but a small portion of the advances made since much of the manuscript was prepared for the press, it leaves the work as a whole somewhat out of date.

While students of the flora of the southeastern United States will find the volume none the less indispensable, the greatest importance which it will have for many taxonomists arises probably from the number of new genera and species and new combinations proposed. The new names alone cover seven pages (pp. 1503–1509), even though numerous technically new names or combinations for subgeneric divisions have been omitted. Descriptions of new species are given in English; in this and various other respects the International Code is ignored. As might have been expected in so large an undertaking, the revisional work upon several of the groups was done by assisting botanists, some of them specialists. Typographically, an exceptionally high standard of excellence has been attained throughout. It is to be hoped that this same standard will be maintained in the companion volume which the author proposes to produce later, dealing with the flora of the country adjacent to the west and extending to the western boundary of Texas.—E. E. SHERFF.

Cytology

The activity of recent years in the field covered by SHARP's[2] important text has necessitated another rewriting. Again the author has summarized a great amount of new material in masterly fashion, and has incorporated it with what went before into an effective unity. Those who make daily use of the results of his labors can but be grateful for the expenditure of time and energy involved in the digestion and organization of an enormous literature. Not only is it cytology proper (or cytology in the older sense) that he has had thus to summarize; preparation of the new edition, more so than in the case of the earlier ones, has required an equal familiarity with the work in experimental genetics. The magnitude of the task can be appreciated by those who find it a man-size job to keep up with the literature dealing with *Drosophila* alone.

A major problem of the author has evidently been to keep his book within the bounds set while including so much that is new. This is evidenced by the following list of new chapters, dealing with subjects not touched, or but very briefly

[2] SHARP, L. W., Introduction to cytology. 3d ed. pp. xiv+567. figs. 230. McGraw-Hill, New York. 1934.

treated, in the previous edition: morphology of the chromosomes; structure of the chromosomes; fragmentation and translocation; reciprocal translocation; heteroploidy; cytogenetics of autoheteroploid plants; cytogenetics of hybrids; cytoplasmic heredity. Also to be included, of course, were new results bearing upon virtually every topic previously treated. Yet the work has been kept within the compass of the previous edition; indeed, the number of pages is slightly fewer.

Many will regret the abridgment thus involved. Some of us would have been glad to see a fuller treatment of many topics and a retention of some discussions that have been deleted. This would have meant a volume of perhaps twice the present size, a volume beyond the reach of students, and probably one that could not be published in this country. But it would have been a better book, and one that only SHARP could write.

One is tempted to speculate as to whether the author did not sometimes question how far he need go in his exposition of the scientific heterozygote known as cytogenetics. He has gone, or so it seems to the reviewer, the full length. At the same time it is probably true that if the field was to be entered, the discussion could hardly have been much briefer. So many, so heterogeneous, and so unorganized as yet are the facts to be considered that a summary organization is out of question. Yet the space devoted is probably disproportionate to the real value of the facts at hand. Certainly the adequate treatment of cytogenetics has meant a less adequate, because greatly abridged, treatment of topics the present knowledge regarding which is probably in reality much more important.

Another problem inevitable in an up-to-date work inheres in the fact that very recent reports of results not yet tested or confirmed must be cited, and it is difficult to cite them at other than their face value. Here enter questions of opinion, and the critic must proceed cautiously. An illustration is the author's apparent acceptance of a few descriptions of the purely intranuclear origin of the spindle in the higher plants. This conception negatives the results of a great number of investigators whose conclusions are not necessarily to be discarded entirely because of their relative antiquity. More skepticism might have been justified in the treatment of some very modern papers dealing with this and other problems upon the basis solely of the Benda fixation. Many cytologists find the Benda fixative especially unreliable except for the purpose for which it was developed, namely, the demonstration of the chondriosomes.

The volume is an excellent and valuable contribution, and the cytological world is once more deeply indebted to its author.—C. E. ALLEN.

Kansas wild flowers

Situated between the regions of eastern and western floras, Kansas has had little in the way of manuals or floras for its students of native plants. This fact makes the recent publication[3] all the more welcome. The home botanist may

[3] GATES, F. C., Wild flowers in Kansas. Kas. State Board of Agric. Rept. 51: 1–295. figs. 448. W. C. Austin, State Printer, Topeka.

now identify many of the plants of the prairie and the plain by the use of this non-technical manual. The task is made the easier by the many clear drawings. If the scale on which each drawing appears on the printed page had been indicated, it would have given the student a better idea of the proportionate size of the plants. The text abounds in keys to families, genera, and species. The classification follows the BESSEYAN system very closely and agrees with RYDBERG in the division of many old genera.

Instances of improved or reformed spelling appear on its pages. There seems no decided objection to "tootht," "brancht," and "groupt," but the use of "Alismataceae" for Alismaceae and "adder's-tung" for "adder's-tongue" will probably seem objectionable to many older botanists. But these are minor objections in a very creditable and useful book.—G. D. FULLER.

Flora of the grasslands

The forests of the east and the mountains of the west have for many years had adequate manuals for the study of their flora. The grasslands that lie between the forests and the mountains have remained comparatively unknown, however, largely because of the lack of anything comprehensive or complete in the way of a manual. A recent volume[4] has removed this deficiency, and once again the botanists are indebted to RYDBERG for undertaking this arduous but needed task. They will all regret that the author received not even the scanty reward of thanks for his labors, as he died while the book was in process of publication. Many will regard RYDBERG's final contribution to botanical science as perhaps his most valuable. In many respects this volume is superior to his Flora of the Rocky Mountains and Adjacent Plains.

In this book the author has apparently followed rather closely the International Rules, although his tendency toward dividing families and splitting genera and species is still in evidence. In addition to the usual collection of keys and descriptions, the present volume has over 500 small illustrations from drawings, which will aid in the identification of many species. The descriptions seem to be concise but adequate, quantitative data for size and number are abundant, ranges are given outside the area included in the manual, and the synonymy is rather complete.

The publishers' work has been well done; the printing is good and the thin paper used has prevented the volume from being too unwieldy.—G. D. FULLER.

Plant names

With the continued neglect of taxonomy in many modern botanical laboratories, BAILEY's recent book[5] should form a welcome addition to botanical bookshelves. In his well known, pleasing style, the author traces the binomial system of nomenclature from the time of LINNAEUS to the present. He also con-

[4] RYDBERG, P. A., Flora of the prairies and plains of North America. pp. vii+969. figs. 600. New York Botanic Garden. New York. 1932. $5.50.

[5] BAILEY, L. H., How plants get their names. pp. vi+209. figs. 12. Macmillan Co., New York. 1933. $2.25.

siders the regulations to be followed in applying names to the many new species that botanical explorations are bringing to light. These considerations are illuminated by appropriate illustrations drawn from his wide experience in this field. Similarly he solves certain intricate problems of synonymy in a demonstration of the working methods of taxonomists. While the book is primarily intended for the use of horticulturists and agronomists, it is equally suited to the needs of botanists who have little familiarity with the rules and usages of botanical nomenclature. A few hours' pleasant reading will clear up many confusing details. These problems include the pronunciation and meaning of hundreds of scientific names. There are a number of interesting illustrations from the herbals of the seventeenth century.—G. D. FULLER.

Principles of plant physiology

The second edition of RABER's volume[6] has just appeared. The text has been enlarged by fuller treatment at certain places, and by additional sections in cases where recent advances warranted the inclusion of new material. The literature lists have been revised to include recent papers and texts. The order of presentation has not been changed, and no new chapters have been added. The approach is through the chemical physiology of the organism, photosynthesis, nitrogen metabolism, respiration, etc. A feature which students will appreciate is the inclusion of photographs of contemporary plant physiologists of Europe and America. Errors which marred the earlier edition have been eliminated. It is a helpful text.—C. A. SHULL.

Recent literature on gymnosperms

Recently an interesting textbook of living and fossil gymnosperms[7] has appeared which seems to be a practical compilation for the use of students. The text is simple and clear and the illustrations instructive although somewhat primitive. The atlas contains 38 plates with figures which look like blackboard drawings. There is nothing original in the text nor in the figures. The bibliography is short and inadequate, consisting primarily of a list of other textbooks. The absence of such an important general text as CHAMBERLAIN's Living Cycads strikes the reader as rather strange. But on account of its simplicity the book recommends itself to the student of plant morphology.

Another publication from the field of gymnospermous plant morphology and taxonomy[8] deals with two Rhaetic-Liassic genera *Cheirolepis* Schimper and *Hir-*

[6] RABER, O. L., Principles of plant physiology. 8vo. pp. xvi+432. Macmillan Co., New York. 1933.

[7] BEAUVERIE, J., Les gymnospermes vivantes et fossiles. Cours de botanique professé à la faculté des sciences de Lyon. Text and atlas in two volumes. 4to. Bosc frères. Lyon. 1933.

[8] HÖRHAMMER, L., Über die Coniferen—Gattungen Cheirolepis Schimper und Hirmeriella nov. gen. aus dem Rhät-Lias von Franken. Bibliotheca Botanica No. 107. E. Schweizerbart'sche Verlagsbuchhandlung. 4to. pp. 33. Illustrated. Stuttgart. 1933. Mk. 22.

meriella Hörhammer. This investigation has been carried out in HIRMER'S laboratory by one of his students. The cuticular analysis method was used and the following technique applied. The stone slabs containing the fossil plants were treated with Schultze's maceration solution. After about 12 hours the rocks had disintegrated to sand and the plants from the surface and the interior of the slabs were entirely preserved and could be seen floating upon the liquid. The description of the two genera and various species is based primarily upon the cuticle and upon the form of the vegetative and some reproductive organs as preserved in the rocks.—A. C. NOÉ.

Plant life through the ages

It is gratifying to see a second edition of SEWARD'S latest work[9] appear only two years after the first one. As was to be expected, the new edition does not differ very materially from the first. There is a list of additional references and a few alterations and corrections throughout the text.

This volume promises to become the standard textbook of plant paleontology in the English language. New editions will always absorb the latest literature and keep the reader informed on the progress of the science. It is admirably fitted to be the indispensable manual of the student as well as of the teacher. It was originally intended to be a book for readers unfamiliar with paleobotany, but it will be read mostly by botanists and geologists who already have some acquaintance with the subject. For a novice's introduction to paleobotany the book mentions too many forms which could be understood only if they were accompanied by illustrations. It seems desirable, therefore, that the number of figures and plates be considerably enlarged. Even the teacher and student of paleobotany would be grateful for such an enrichment of the book.

The lucidity and beauty of the author's diction and the rich background of his information are bound to guarantee continued success for this extremely useful book.—A. C. NOÉ.

Origin of cultivated plants

A very comprehensive treatment of the origin of cultivated plants[10] divides the subject matter into two parts, one general and the other special in character. The former contains chapters on methods of phylogenetic research and on the differences between wild and cultivated forms. For the botanist it is rather novel to see under the section on phylogenetic research a subchapter on historical-philological methods and linguistics. The study of the origin and migration of plants cultivated by man is helped by an investigation of plant names in different languages.

Using the resources of botany, anthropology, and philology, a full account is given of the history of cultivated plants since Neolithic times.—A. C. NOÉ.

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[9] SEWARD, A. C., Plant life through the ages. 2d. ed. 8vo. pp. xxi+603. figs. 139. University Press. Cambridge; Macmillan, London. 1933. $8.00.

[10] SCHIEMANN, E., Entstehung der Kulturpflanzen. Handbuch der Vererbungswissenschaft. Vol. III. pp. ix+377. figs. 96. Bornträger, Berlin. 1932. RM 50.

Taxonomic studies

Two interesting monographs appeared recently, one of which deals with a subdivision of the genus *Veronica*[11] and the other with the geographical and morphological features of *Aster alpinus* L.[12]

Both papers show well the modern type of ecologic-taxonomic investigation.—A. C. Noé.

New paleobotanical series

The well known paleontological publication Palaeontographica has established a subdivision devoted exclusively to paleobotany. It is edited by Max Hirmer in Munich and counts among its associate editors such names as Paul Bertrand, W. Gothan, T. G. Halle, W. J. Jongmans, R. Kräusel, J. Walton, H. Weyland, and G. R. Wieland. Its first number[13] appeared recently and contains contributions by Kräusel and Weyland on the flora of the Middle Devonian in Bohemia and by Hirmer on the reconstruction of *Pleuromeia sternbergi* and on the morphology of Lycopodiales in general. Text and illustrations are excellent but the price is too high.

Every volume of this section will comprise six numbers. Only original articles are to be published in this series. It promises to become a very important collection of valuable monographs. Section A is devoted to paleozoology. Formerly only one series existed which contained both lines of paleontology. In this common series 77 volumes have been published already and the enterprise is old and well established.—A. C. Noé.

Cultivated conifers

Any book from Bailey's pen commands the attention of both botanists and horticulturists, for no one is better able to speak with authority in the two fields. Within the pages of this new volume[14] the botanist will find a critical and able discussion of the principles of classification and their application to the two families of the conifers. Synonyms are given and many of the troublesome problems of nomenclature considered in detail. In addition there are good descriptions of the different species with a careful consideration of geographic range. The illustrations are useful and a number of the plates are from beautiful photographic studies.

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[11] Harle, A., Die Arten und Formen der Veronica-Sektion Pseudolysimachia Koch auf Grund systematischer und experimenteller Untersuchungen. Bibliotheca Botanica No. 104. E. Schweizerbart'sche Verlagsbuchhandlung. 4to. pp. 86. Illustrated. Stuttgart. 1932. RM 47.

[12] Onno, M., Geographisch-morphologische Studien über *Aster alpinus* L. und verwandte Arten. Bibliotheca Botanica No. 106. E. Schweizerbart'sche Verlagsbuchhandlung. 4to. pp. 83. Illustrated. Stuttgart. 1932. RM 38.

[13] Palaeontographica. Vol. LXXVIII. Section B. Paleophytologie. Edited by Max Hirmer. Lieferung 1–2. E. Schweizerbart'sche Verlagsbuchhandlung. 4to. pp. 56. pls. 7. Illustrated. Stuttgart. 1933. RM 68.

[14] Bailey, L. H., The cultivated conifers in North America. pp. ix+404. pls. 48; figs. 114. Macmillan Co., New York. 1933. $7.50.

Both botanists and horticulturists will be interested in the many varieties that are recognized and given brief descriptions. For example, not less than ten varieties of Colorado blue spruce are noted, while the different varieties of the Norway spruce number fifty. One is surprised to find how largely Japan and China have contributed to our cultivated trees.—G. D. FULLER.

British economic grasses

At best the identification of grasses is a matter of considerable difficulty. Especially is this true of those forms occurring as a part of grazed lands where frequently only vegetative structures are available for study. The present work[15] is based on a detailed examination of the internal structures of the leaves of all the chief species of British grasses, supplemented with a critical study of other vegetative characters of diagnostic significance. More than fifty species are illustrated and their pertinent microscopic characters enumerated. There are brief keys based on anatomical details and vegetative characters. It may not be too much to hope that there may be more contributions of similar kind.—E. J. KRAUS.

The genus Diaporthe

A recent volume by WEHMEYER[16] is a taxonomic treatment of the genus *Diaporthe* and its allied genera, *Diaporthopsis*, *Apioporthe*, *Diaporthella*, and *Cryptodiaporthe*. There are keyed and described 71 species of *Diaporthe*, 6 species of *Diaporthopsis*, 8 species of *Apioporthe*, 3 species of *Diaporthella*, and 19 species of *Cryptodiaporthe*. One section of the volume is devoted to doubtful species of these genera, another to excluded species, and a large section to species not seen. The volume should prove of interest and use, not only to mycologists, but to pathologists interested in the diseases incited by the perfect and imperfect stages of the ascomycetes treated in this volume.—G. K. K. LINK.

Phytopathological and botanical research methods

The volume by RAWLINS[17] on phytopathological and botanical research methods is a contribution to botanical teaching and research which will be welcomed by all who attempt either the teaching or the learning of current phytopathological technique.

The volume deals essentially with microbiological techniques applied to phytopathogenic viruses, bacteria, and fungi. In addition to the microbiological, the book deals briefly with histological, microchemical, and biometrical techniques. Possibly this is the reason for the phrase "botanical research methods"

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[15] BURR, SYDNEY, and TURNER, DOROTHY M., British economic grasses: their identification by the leaf anatomy. pp. 94. figs. 111. Arnold & Co., London; Longmans Green, New York. 1933. $3.75.

[16] WEHMEYER, L. E., The genus *Diaporthe Nitschke* and its segregates. pp. vi+349. pls. 18. University of Michigan Press, Ann Arbor. 1933.

[17] RAWLINS, T. E., Phytopathological and botanical research methods. pp. ix+147. figs. 3. J. Wiley & Sons, New York. 1933.

in the title. Unfortunately one misses a chapter devoted to the techniques used in studying the rôle and influence of internal factors or of non-living environmental factors as direct and indirect pathogenic factors in non-infectious diseases; or of the indirect rôle and influence of internal as well as living (aside from the rôle of insects in virus diseases) and non-living external factors in infectious diseases. Possibly obligatory limitation of size of the volume demanded omission of these basic phytopathological techniques.

The growing field of virus diseases is recognized by a brief chapter. The bibliography of 960 titles is a valuable addition to the volume.—G. K. K. LINK.

Tradescantia

A brief volume has just appeared[18] in which is reviewed the rôle that the cells of *Tradescantia*, notably those of its stamen hairs, have played in the study of phytocytological problems during the past hundred years. Recent observations of the author are referred to, with a promise of more detailed discussion later. The recent literature is reviewed more exhaustively than the older, the former furnishing the key to the latter in so far as this is not listed. The review focuses attention upon the fact that *Tradescantia* cells have been used to a truly amazing extent in the study of nuclear, chromosomal, cytoplasmic, membranal, vacuolar, explantational, and other cellular problems.—G. K. K. LINK.

Dictionary of horticulture

Horticulturists and botanists alike will welcome this reissue of Hortus.[19] Here in a single volume is an annotated inventory of the main varieties of plants now in more or less general cultivation in the United States and Canada. Although the references are brief, they provide a wealth of helpful information with respect to methods of cultivation and nomenclature, which follows the system of the International Rules of 1905 and 1910.

It is the expressed hope of the authors, in which they will certainly be joined by all who must deal with cultivated plants, that volumes similar to this one will be revised, brought down to date, and reissued frequently. No other adequate method exists whereby an account may be kept of the cultivated flora.—E. J. KRAUS.

Virus diseases

The study of the effects of viruses in plants has led to such an abundance of empirical data that a summary statement has become necessary. In a recent volume[20] SMITH and BROOKS have ably assembled and summarized the enormous

[18] KÜSTER, ERNST, Hundert Jahre *Tradescantia*. pp. 36. figs. 7. Fischer. Jena. 1933.

[19] BAILEY, L. H. and ETHEL Z., Hortus: a concise dictionary of gardening, general horticulture and cultivated plants in North America. pp. 652. pls. 16; figs. 22. Macmillan Co., New York. 1930. Reissued 1934 at reduced price of $5.00.

[20] SMITH, K. M., and BROOKS, F. T., Recent advances in the study of plant viruses. pp. xii+423. figs. 67. P. Blakiston Son & Co., Philadelphia. 1934.

body of literature, much of it contradictory, which has grown out of the study of the diseases incited in plants by the invisible, supposedly living, agents which are designated as viruses. The volume should prove of great use to investigators in the field of virology as well as to phytopathologists and botanists in general. Some clarifying discovery and unifying generalization are necessary to bring out any underlying and hidden relations that may pertain to virus diseases and their incitants. The authors are fully aware of this, stating: "Not much further progress is likely to arise from continued study of symptomatology to which too much attention has been given in the past." It is not so much that excessive attention has been given to this phase, but rather that not enough attention has been given to other aspects of the problem.—G. K. K. LINK.

Recent advances in plant physiology

The necessity of printing a second edition of BARTON-WRIGHT'S[21] summary of progress in plant physiology in recent years attests its usefulness and popularity. There are some changes in organization, and some omission of material formerly included. The soil relations have been practically omitted, and there are now nine chapters instead of six, mainly because growth phenomena are given three chapters instead of one. The chapter headings are as follows: absorption of water and transpiration; carbon assimilation; nitrogen metabolism; the raw materials of plant nutrition; translocation; respiration; growth (three chapters). The revision has been accomplished with a slight reduction in the size of the volume.

It is a well written account of the recent progress in this field, and will be especially useful to students who need assistance in following the general trend of development of the subject, and in gaining a critical viewpoint with reference to recent work. The former edition won a wide circle of friends for the gifted young author, and these friends will welcome and extend the use of the new edition. —C. A. SHULL.

SIXTH INTERNATIONAL BOTANICAL CONGRESS

Amsterdam, September 2–7, 1935

The Organizing Committee of the VI International Botanical Congress has been asked to change the dates of this Congress. The Committee has now decided that the Congress will meet at Amsterdam (Holland), September 2–7, 1935.

A first notice regarding this Congress has been sent out to a number of addresses; for additional copies apply to the secretary, Dr. M. J. SIRKS, Wageningen, Holland.

[21] BARTON-WRIGHT, E. C., Recent advances in plant physiology. 2d ed. 16 mo. pp. x+341. figs. 54. P. Blakiston Son & Co., Philadelphia. 1933. $4.00.

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THE

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June 1934

AN IMPROVED METHOD FOR THE PURIFICATION OF CHLOROPHYLLS *A* AND *B*; QUANTITATIVE MEASUREMENT OF THEIR ABSORPTION SPECTRA; EVIDENCE FOR THE EXISTENCE OF A THIRD COMPONENT OF CHLOROPHYLL

F. PAUL ZSCHEILE, JR.[1]

(WITH PLATE X AND THREE FIGURES)

Introduction

The existence of two components of chlorophyll was first announced as a logical deduction from experimental data by STOKES (8) in 1864. He used the method of fractionation between solvents (9), carbon bisulphide and ethyl alcohol in his case, to separate the two green components of chlorophyll from each other and from the yellow pigments, carotene and xanthophyll, all of which he differentiated spectroscopically. The existence of two components of chlorophyll was concluded incorrectly two years earlier, in 1862, by SIMMLER (5) from his observation that chlorophyll fluoresced in both the red and the green regions of the spectrum. This same observation had been made in 1852 by STOKES (7), who studied the fluorescence of alcoholic leaf extracts visually through a prism. SORBY (6), using fractionation methods with carbon bisulphide and aqueous ethyl alcohol, distinguished between three different chlorophylls: (1) blue chlorophyll which formed a bluish green solution, (2) yellow chlorophyll which formed a yellowish green solution, and (3) chlorofucin.

[1] National Research Council Fellow in the Biological Sciences.

His methods, although not clearly described, may be criticized severely because they involve treatment of the chlorophyll preparation with heat, alkali, and perhaps impure solvents. Moreover, he used algae in much of his work and in no single group of algae (brown, red, or green) did he find all three components.

A new method of chlorophyll component separation was introduced by TSWETT (10) in which a petroleum ether, benzene, or carbon bisulphide solution of crude chlorophyll was filtered through a column of calcium carbonate or sugar. He states that carbon bisulphide is the most efficient. In this way a chromatogram was obtained, and the different pigments were found separated in zones of the adsorbent. These zones were separately extracted with suitable solvents and solutions of the various pigments were thus obtained. In addition to a number of xanthophylls in various yellow zones, TSWETT found two chlorophyll pigments in adjacent zones. The upper zone was dark olive green in color and contained chlorophyllin β; the lower one was dark blue-green and contained chlorophyllin α. They correspond to the chlorophylls *b* and *a* of WILLSTÄTTER, whose work will be considered later. TSWETT (11) studied these green pigments spectroscopically with the methods then available.

Thus far, neither the chlorophyll components nor their mixture had been prepared in a state free from colorless impurities and yellow pigments. Chlorophyll was isolated from such impurities by WILLSTÄTTER *et al.* (13) in a pure and unchanged state and the components were supposedly separated from each other, but, as will be shown later, this separation was incomplete. By making certain additions to their method, components *a* and *b* have been completely separated from each other and isolated in the pure state. Evidence will be presented of the possibility of the existence of a third component.

Throughout this discussion, the term chlorophyll refers to the green (and blue) fat-soluble pigments extracted from fresh green plant tissue, and is not to be confused with "la chlorophyll naturelle" of LUBIMENKO (3) or the "phyllochlorins" of MESTRE (4). The latter refer to the *in vivo* green pigments composed of the chlorophyll components plus whatever proteins, lipoids, or yellow pigments which may be in combination with them in the living chloroplast.

Experimentation

In the present investigation the preferential adsorption method of TSWETT was combined with WILLSTÄTTER'S method of fractionation between immiscible solvents to isolate chlorophyll *a* in a pure state, free from component *b*. A fractional precipitation method was used to prepare component *b* free from *a*. Inert and specially purified solvents were used throughout, no chemical reactions were employed, and the chlorophyll was never heated above room temperature. The test of purity which is most reliable is the absorption spectrum of the component considered. This is true because of the great accuracy and precision with which the absorption of light at any desired wave length may be measured by use of the spectro-photoelectric method (16) developed recently and applied to the chlorophyll pigments.

METHOD OF ISOLATION OF CHLOROPHYLL COMPONENTS *a* AND *b*

Following is a brief outline of the method used for the isolation of pure chlorophylls *a* and *b* from fresh leaf material. This method is based on results from fifty-five component separations and is the shortest method found by the writer to give the most efficient separation of the chlorophyll components. That part of the method above the heavy line of the chart follows the procedure of WILLSTÄTTER and STOLL very closely, although some variations are introduced which are important to secure a complete isolation of *a* and *b*. It must be made clear that if WILLSTÄTTER'S procedure is followed exactly, especially in regard to certain details, one obtains the same results as his, and the preparations have the same absorption spectra as those reported by WILLSTÄTTER and STOLL (13). Since their method may be extended and modified to produce components with very different absorption spectra, separations were made under widely different conditions and many steps of the procedure were varied in order to determine whether or not these new results were due to experimental error.

The steps of the method are discussed in detail as follows. The percentage composition of all solvents used in the preparative procedure is expressed on the basis of volume unless specifically stated otherwise.

METHOD OF ISOLATION OF CHLOROPHYLL COMPONENTS *A* AND *B**

Ground fresh leaves
(Extract with acetone)
I↓
Acetone extract
(Add to petroleum ether and wash with H_2O, 80% acetone, and H_2O)
II ← → IIa

II: Petroleum ether layer containing chlorophyll, carotene, and xanthophyll (Wash with 80% CH_3OH)

IIa: Aqueous layer containing acetone, water-soluble pigments (as anthoxanthins) and colorless impurities

III ← → IIIa

III: 80% CH_3OH containing xanthophyll

IIIa: Petroleum ether solution containing chlorophyll, carotene, and some xanthophyll (Wash with H_2O)

IVa ← → IV

IVa: Petroleum ether suspension of *a*; carotene and xanthophyll in solution (Filter on talc and extract with ether)

IV: H_2O+CH_3OH

V ← → Va

V: Petroleum ether solution of carotene and xanthophyll

Va: Chlorophyll $(a+b)$+small amounts of xanthophyll and carotene (Transfer to petroleum ether, wash with H_2O, 85%, and 90% CH_3OH)

VI ← → VII

VI: Petroleum ether solution rich in *a* (Wash with H_2O)
← → VI 1

H_2O+CH_3OH

Petroleum ether suspension rich in *a* (Filter on talc and extract with ether)
↓VI 2
Ether solution rich in *a*
(Add to petroleum ether)
↓VI 3†
Ether-petroleum ether solution rich in *a* (Filter on thick talc filter) Last traces of yellow pigments removed in filtrate
↓VI 4
Green and *blue* zones (Separate mechanically)
VI 5 ← →

Green talc with adsorbed $a+(b$ or "c"?) (Extract with ether)
↓VI 6
Green ether solution of mixture of $a+(b$ or "c"?)

Blue talc with adsorbed *a* (Extract with ether)
↓VI 7
Blue ether solution of *a*

VII: CH_3OH solution rich in *b* (Wash with petroleum ether)
↓VII 1 → Petroleum ether solution rich in *a*
CH_3OH solution rich in *b* (Transfer to ether; wash with H_2O)
↓VII 2 → H_2O+CH_3OH
Ether solution rich in *b* (Add to petroleum ether and filter on talc)
↓VII 3 → Petroleum ether solution of $a+b$ +last traces of xanthophyll
Component *b*, precipitated on talc (Extract with ether and filter)
↓VII 4
Ether solution of *b*

* The distribution of the yellow pigments between solvents was obtained by private communication with ELMER S. MILLER.

† Steps VI 3–7 are additions to the procedure of WILLSTÄTTER and STOLL.

STEPS OF PURIFICATION METHOD

I. From 1.0 to 1.5 kg. of fresh green leaves are ground rather coarsely with a food grinder and collected in an enameled pan. Within two or three minutes after the grinding process, the juicy pulp is treated with 500 cc. of acetone and thoroughly stirred in a Pyrex beaker. A 1.5 ft. square of washed muslin is spread over the enameled pan and the mixture is placed on this cloth. As much as possible of the liquid is squeezed out by hand. More of the liquid is then extracted by the use of a hand press, lined with tin. The pulp is again extracted in the same fashion with 500 cc. of acetone. Most of the water is removed by these two extractions, the first of which is yellow and the second yellowish green. They contain very little chlorophyll and a large amount of yellow anthoxanthins and colorless substances, and are therefore discarded.

The press cake from the second extraction is reground and the meal is then treated with 400 cc. of acetone, stirred, and placed in the cloth. As in the first treatment, the liquid is removed from the plant meal, first by hand and later by the press. The deep green extract must be filtered by suction to remove solid particles of plant meal. The extraction process is repeated several times. The third, fourth, and fifth extracts contain most of the chlorophyll. These acetone solutions are saturated with chlorophyll and some of it usually precipitates on the filter paper with the solid sediment of leaf tissue particles. When this occurs, 25 to 50 cc. of acetone may be poured through the filter to remove the chlorophyll. A total of about eight extractions is usually necessary to remove the chlorophyll to such an extent that the meal becomes gray and the extract very light green.

II. The acetone extracts are filtered separately and added to a mixture of 1.0 liter of petroleum ether (b.p. 35–55° C.) and 100 cc. of acetone in a 4-liter glass separatory funnel. The extracts are poured through a small funnel in such a manner that they flow slowly down the side of the separatory funnel. After the first extract has been added and the contents of the separatory funnel have been rotated gently, some chlorophyll remains in the acetone-water layer, which is drained off and discarded. Care must be taken that the mixture is not too vigorously agitated, especially at this point, in order that emulsion formation may be avoided. After the second addition of extract to the petroleum ether, the added acetone usually mixes easily with the petroleum ether and forms a clear solution that is bright red by transmitted light. The volume of the acetone-petroleum ether mixture increases to 2.5–3.0 liters by addition of the extracts.

A small electric filament lamp is convenient as a means of examination of solutions and suspensions throughout the whole separation process.

The lamp is held close to the solution on the side of the container opposite the eye. The nature of the appearance of the filament is a good indicator of the chlorophyll's condition in the liquid. When the filament appears red and well defined, the chlorophyll is in true solution and no emulsion is present. An emulsion has a cloudy appearance. Precipitation of the chlorophyll causes disappearance of the red color and formation of black particles and the suspension becomes opaque.

IIa. While the mixture is rotating, water is run gently down the sides of the funnel from a second funnel above the large one. Most of the acetone goes into the aqueous layer, accompanied by colorless and some yellowish impurities. When about 2 liters of water have been used in this manner, emulsions begin to form and the washing with water is discontinued. The petroleum ether layer is then washed twice with 150 cc. of 80% acetone and again with water until emulsions start to form.

III. After most of the acetone has been washed from the petroleum ether layer with water, the petroleum ether solution of chlorophyll, carotene, and xanthophyll is washed five times with 200 cc. of 80% methyl alcohol to remove most of the xanthophyll. The first two of these methyl alcohol washings are dark green in color, the last one light greenish yellow.

IIIa. The petroleum ether solution of chlorophyll is later to be washed with 85% methyl alcohol as part of the separation of *a* and *b*. Since the carotene is present, emulsions form rather easily with 85% methyl alcohol; therefore it is desirable to remove the carotene at this point.

IV. The petroleum ether solution of chlorophyll, carotene, and 15–20% of original xanthophyll is washed cautiously with water, which removes the methyl alcohol.

IVa. The chlorophyll precipitates when all of the methyl alcohol is removed. The particles can be seen with the aid of the electric lamp; the lamp filament loses its red color when seen through the suspension. When complete precipitation occurs, the fluorescence disappears and the suspension becomes very opaque to light. The presence of yellow pigments makes complete precipitation difficult.

V. The suspension, after drying over anhydrous sodium sulphate, is filtered through a layer of talc about 3.0 cm. in thickness. This talc filter is made on a Buchner funnel of 15 cm. diameter and 5.5 cm. depth. The talc layer is made by wetting the talc with petroleum ether and applying gentle suction. The talc is pressed down with a large spatula while suction drains off the petroleum ether until the talc forms a hard and fairly dry layer. The precipitated chlorophyll forms a thin black layer on top, which needs slight stirring occasionally to facilitate filtration. Usually the

chlorophyll is not completely precipitated and some of it penetrates the talc a short distance. Several washings with petroleum ether remove all but a trace of the carotene from the top black layer. Some xanthophyll precipitates with the chlorophyll.

Va. After the black layer of chlorophyll has become fairly dry by suction, it is scraped off with a spatula and immediately placed in anhydrous ether.[2] A complete stirring of this mixture insures solution of all of the chlorophyll in the ether. The talc is removed by filtration and an ether solution of chlorophyll plus some xanthophyll and a trace of carotene results. This is evaporated quickly to dryness by means of an electric fan, and heat is thus avoided.

VI–VII. The chlorophyll is dissolved in as small a volume of ether as possible and added to 500 cc. of fresh petroleum ether. Two washings with 200 cc. each of 80% methyl alcohol remove most of the ether and are discarded. The petroleum ether solution is then extracted twenty times, each time with 500 cc. of 85% methyl alcohol. Finally, three extractions with 500 cc. of 90% methyl alcohol are made. Before use, the methyl alcohol solutions are saturated with petroleum ether, and to prevent allomerization, 0.01 gm. of oxalic acid per liter is added. After each methyl alcohol addition has been made the whole mixture is rotated gently to provide more complete contact between the two phases. According to WILLSTÄTTER and STOLL, who extracted only sixteen times with 85% methyl alcohol, these washings remove most of component *b* from the petroleum ether layer and the 90% methyl alcoholic washings extract the last traces of *b*. The petroleum ether solution is rich in chlorophyll *a* and usually contains a small amount of yellow pigments.

VI 1. This petroleum ether solution is washed with water until the methyl alcohol has been removed and complete precipitation occurs. Thick layers of the mixture are now opaque and particles of chlorophyll are easily visible. All fluorescence has disappeared and the suspension is black.

VI 2. After drying with sodium sulphate, the suspension is filtered on a 2 cm. layer of talc and the precipitated chlorophyll is washed several times with petroleum ether. The top black layer is mechanically removed and extracted with ether. If a small amount of the chlorophyll penetrated the talc this may be discarded, as the amount lost will be small.

VI 3. The green ether solution is evaporated to dryness by an air current. The water of condensation may be easily poured off when the ether has completely evaporated.

At this point, the method of WILLSTÄTTER and STOLL ends, this prepa-

[2] Ether signifies di-ethyl ether unless specifically designated petroleum ether.

ration being designated as component *a*. Further to purify this preparation, the pigment is dissolved in 70 cc. of ether and added to 300 cc. of petroleum ether (b.p. 35–55° C.). No chlorophyll is precipitated and a bluish green solution results.

VI 4. This intensely fluorescent solution is filtered through a thick talc filter, 4.5 cm. thick, made as described in step V. No precipitated layer forms on top of the filter and the chlorophyll penetrates the talc, forming a chromatogram (pl. X, B). After two washings with petroleum ether, the talc is moderately dried by suction and gradually removed from the top with a spatula. The last traces of yellow pigments are removed in the filtrate.

VI 5. A green zone (pl. X, B 1) is found in the top portion of the talc layer. The upper fifth of this green zone is lighter in color than the remainder. Below the green zone is a *blue* zone (pl. X, B 2). The thickness of the green layer is from one-third to one-half that of the total colored talc layer. If the thickness of the talc is not sufficient to adsorb the amount of chlorophyll present in the solution, some of the blue fraction will be found in the last portion of the filtrate. Provided the solution has been properly prepared in regard to ether concentration, there is a sharp line of demarcation between the green and blue zones. Should the ether concentration be too high, however, a good separation of the blue from the green pigments is not obtained and the chlorophylls will not be adsorbed on the talc; if it is too low, the color zones will be too thin to permit efficient separation or some chlorophyll will precipitate as a black deposit on top of the talc. To obtain these two regions of talc uncontaminated with each other, a middle section, which includes the line of demarcation, is discarded. With care this discarded section may be made quite small. It is especially necessary that the blue layer be uncontaminated with the green.

VI 6, 7. The separated portions of talc are immediately placed in anhydrous ether, thoroughly stirred, and filtered quickly. The chlorophyll is removed as quickly as possible from the talc with fresh ether and the washed talc is discarded. From 300 to 400 cc. of ether is required for each talc portion. The solutions are evaporated to dryness by an air current. Water of condensation collects beneath the chlorophyll, owing to the rapid evaporation of ether. This water layer prevents the chlorophyll's adherence to the glass, which in turn facilitates the removal and pulverization of the pigment.

The green solution contains component *a* plus one or more other components. These will be considered later in more detail. The blue ether so-

lution, if sufficiently concentrated, is pure *blue* by transmitted light and contains pure chlorophyll *a*.

PURIFICATION OF COMPONENT *b*

VII 1. The 85% methyl alcohol extracts are washed with petroleum ether to remove chlorophyll *a*. To each of the first five extracts, 120 cc. of methyl alcohol is added to raise the concentration to 90%. After the fifth extract the methyl alcohol addition is decreased 12 cc. each time. These extracts are added in pairs to 120 cc. of petroleum ether and washed by gentle rotation to remove *a*, a large part of which goes to the petroleum ether layer. The carotene is completely removed by the petroleum ether. The twenty 85% methyl alcohol extracts are divided into three groups: (1) extracts 1 to 4; (2) extracts 5 to 10; and (3) extracts 11 to 18. The remaining two 85% methyl alcohol extracts contain too little chlorophyll *b* for recovery and the 90% methyl alcohol extracts contain too much chlorophyll *a* for efficient isolation of *b*.

VII 2. The groups of extracts are mixed with 500 cc. lots of ether and sufficient water is added to cause a separation into two phases. The aqueous layer, which is light yellow or colorless, is discarded. The ether solutions are washed with water to remove methyl alcohol and are then evaporated to dryness with an air current.

VII 3. These preparations of component *b* are each dissolved in 13 cc. of ether, and 100 cc. of low-boiling petroleum ether (b.p. 30–35° C.) is added. The result is a true solution (red by light transmitted through thick layers and no particles visible) and remains clear for about 15 minutes if allowed to stand until part of the ether has evaporated. An air current will hasten the precipitation of chlorophyll *b*, which may be closely followed by observing the formation of precipitated particles. When approximately two-thirds of the total pigment (estimated visually by intensity of transmitted light) has precipitated, the suspension is filtered on a thin layer of well compressed talc in a small Buchner funnel. The precipitated portion, consisting of chlorophyll *b*, is carefully removed from the filter, including as little talc as possible. The dissolved portion contains xanthophyll, component *a*, and some *b*, which penetrate the talc filter and are discarded.

VII 4. The precipitated *b* is dissolved from the talc with ether. The solution is filtered and evaporated to dryness with an air current. Of the three resulting preparations of *b*, only the third (extracts 11 to 18) is usually pure. When the phase test (saponification of an ether solution with methyl alcoholic potassium hydroxide) is applied, and water is added after

complete saponification, the ether layer is colorless if all xanthophyll and carotene have been removed. In the case of *b* from extract group 3, the ether layer is colorless, but that from group 2 is usually slightly yellow and that from group 1 is distinctly yellow. If the chlorophyll from groups 1 and 2 were reprecipitated by the preceding method, probably the xanthophyll could be completely removed. Since this reprecipitation is very wasteful of *b*, larger quantities should be used than may be obtained from 1 kg. of fresh leaves.

Discussion of chlorophyll chromatograms

Evidence from more than one type of data points toward the existence, in an acetone extract from fresh green leaves, of more than two components of chlorophyll. The green component which is called "component *c*" in this paper, and is found in the green layer ("fraction c") of the talc in step VI 4, may be a third component of chlorophyll. Evidence will be presented here and later in the paper that component *c* has some properties similar to *a* and others similar to *b*. In the green fraction c there may be more components than component *c*, in addition to *a*.

Some chlorophyll was extracted with acetone from fresh grass, transferred to 1 liter of petroleum ether, and washed five times with 450 cc. of 75% methyl alcohol to remove most of the xanthophyll. After precipitation by washing with water, ether was added until the fluorescence reappeared. This mixture, containing *a*, *b*, component *c*, carotene, and doubtless some xanthophyll and colorless impurities, was filtered on talc in a glass Gooch funnel. The chromatogram (pl. X, A) was washed with fresh petroleum ether to remove carotene and perhaps part of the xanthophyll.

Four distinctly different zones of approximately equal thickness were found in the talc. They were separately extracted with ether and the phase test was applied to each solution. The upper zone, 1, was very light grayish green and contained mostly colorless impurities with only a small amount of chlorophyll. The very thin darker green layer of zone 1 was not isolated, but was discarded with the remainder of zone 1. The ether solution (from zone 1) gave a yellowish brown phase test. Zone 2, dark green in color, gave a brown phase. Zone 3 was lighter green than the second. Zone 4 was pure *blue* and contained component *a*. The ether solutions of these lower

two zones gave yellow phases. These phase tests indicated that *b* was present mostly in the dark green zone 2, mixed with *a*. Of the four zones, only the lower one, zone 4, contained a pure substance, as determined by color and phase test.

The ether solutions from the zones formed in step VI 4 give yellow phase tests. When the ether-petroleum ether solution of the green and blue pigments ((VI 3) is filtered through the talc, the green is adsorbed in a layer above the blue (pl. X, B). As more solution is filtered through the talc these colored zones widen. The blue layer always precedes the green layer. Probably two processes occur during the filtration: (1) the green displaces the blue, which is readsorbed lower down in the filter; (2) the green is adsorbed upon or beside the adsorbed blue on the talc surface but does not cover all of the adsorbed blue because the ratio of green to blue is too small. When the green layer (pl. X, B 1) is extracted separately with ether and the filtration of step VI 4 repeated, a blue zone is formed which is much thinner in comparison with the green zone than was the case in the first filtration. A third filtration of the green fraction produces a blue zone having a depth equal to only about 5% of the total.

The separation of the blue and green fractions by differential adsorption on talc is most efficient from an ether-petroleum ether solution which is red by transmitted light and strongly fluorescent. Twenty per cent ether (by volume) is the optimum concentration. When methyl alcohol is used instead of ether, and if exactly the proper amount of methyl alcohol is present, a good separation occurs. The separation is more difficult to regulate and control in this case, as a slight excess or insufficiency of methyl alcohol causes too little or too much adsorption in the talc to produce a good separation. The relative thickness of a zone is not an exact indication of the relative amount of chlorophyll adsorbed in that zone. The colors and contrasts of the zones fade rapidly upon drying.

Other solids were studied on a small scale as adsorptive agents for the separation of *a* and fraction c to determine to what extent the adsorptive surface might be specific for this separation. Stock materials were used and no special washings were applied for purification purposes. Powdered sucrose, powdered calcium carbonate (c.p.), and infusorial earth (fairly white) gave separations of the blue and green fractions equally as definite as those obtained with talc. Magnesium oxide was not so satisfactory, since the lower layer was blue-green and the upper one gray. Kaolin

(rather dark colored) adsorbed the chlorophyll more strongly than did the other solids used. The zones were thinner and their color differences were somewhat masked by the color of the kaolin.

Absorption spectra of components *a* and *b*

The absorption spectra of the chlorophyll components *a* and *b* in ether solution were studied quantitatively by a photoelectric method developed by ZSCHEILE, HOGNESS, and YOUNG (16). The resulting light absorption curves are presented in figure 1. Measurements were taken at intervals of 25 Å. The spectral regions isolated by the monochromator varied in width from 6 Å. at λ 3950 Å. to 13 Å. at λ 7000 Å. Solutions were changed after every sixth reading (at intervals of 150 Å.) to avoid photodecomposition of the chlorophyll.

Values of α, the light absorption coefficient defined by BEER's law,

$$I_x = I_0 \times ^{-\alpha c x}$$

$$\alpha = \frac{\log \frac{I_0}{I_x}}{cx},$$

are plotted as ordinates of the curves. In this equation,
I_0 = intensity of light transmitted by the solvent-filled cell.
I_x = intensity of light transmitted by the solution-filled cell.
x = thickness of absorption cell in cm. (4.25 cm.).
c = concentration of chlorophyll in gm. per liter.
The logarithm is to the base 10; α is expressed in liters per gram centimeter.

A concentration of 0.0007 gm./liter was used in the blue and red regions of the spectrum where absorption is greatest. In the green and yellow regions a concentration of 0.0150 gm./liter was used. The ether was freshly distilled over sodium and the solutions were kept in the dark. The values of α at the maxima and minima of absorption were accurately determined with solutions whose concentrations were known with an accuracy of 1.0%. The ratio $\frac{I_0}{I_x}$ varied between 1.5 and 3.0. These values of α at the maxima and minima were determined with a precision of better than 0.5%. α was calcu-

lated from BEER's law for all wave lengths measured, and when these values, derived from measurements at different concentrations, were

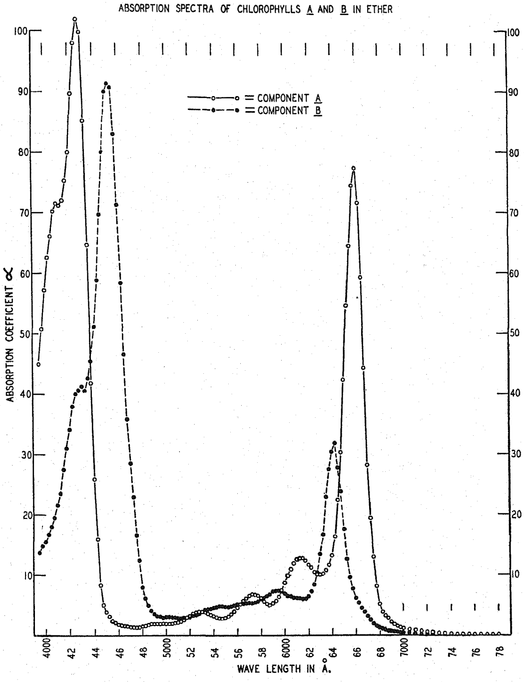

FIG. 1

plotted against wave lengths, it was found that the overlapping portions agreed very closely. This demonstrates that BEER's law is obeyed at these concentrations in ether solution. At the maxima and

minima the accuracy of α is 1.0% or better and at other points on the curves it is 2.0%. The wave lengths of the maxima are accurate to 10 Å. The values of α and λ for the maxima are presented in table I.

These absorption spectra of the chlorophyll components, prepared by the improved technique just described, will now be compared with those obtained by WILLSTÄTTER and STOLL (13, pls. VI, VIII). According to WILLSTÄTTER, the absorption spectra of the methyl chlorophyllides, as observed visually, are the same as those of the pure components.

TABLE I

ABSORPTION MAXIMA OF COMPONENTS *a* AND *b* IN ETHER SOLUTION (LISTED IN ORDER OF DECREASING INTENSITY)

CHLOROPHYLL *a*		CHLOROPHYLL *b*	
WAVE LENGTH IN Å.	α	WAVE LENGTH IN Å.	α
4275	102	4525	91.3
6600	77.4	4300	41.2
4100	71.5	6425	31.8
6125	12.7	5925	7.36
5725	6.75	5675	5.27
5275	3.86	5475	4.77
4975	1.93	5025	2.99

In the spectrum of *a* there is no trace of a band at λ 4500–4600 Å., such as WILLSTÄTTER reported. Since the chief absorption band of *b* has a maximum at λ 4525 Å., it would seem that WILLSTÄTTER'S *a* was contaminated with *b* or with some other substance which absorbs strongly in this region. The smaller band at λ 4100 Å. could not have been detected by WILLSTÄTTER'S method; otherwise the absorption bands of *a* have about the same order of intensity as that reported by WILLSTÄTTER.

In the spectrum of *b*, it is noted that the 6425 Å. band is much less intense than the 6600 Å. band of *a*. The 6425 Å. band has a steep slope toward the red side and shows no trace of a band at λ 6600 Å. WILLSTÄTTER'S *b* has an intense band at λ 6600 Å., which would be caused by an impurity of *a*. Also his *b* shows a weak band at λ 6150 Å. This would be caused by an impurity of *a*, which has a strong band at that same wave length. The 4300 Å. band of *b* is not caused

by an impurity of *a*, whose most intense band occurs at this wave length. This is evident from consideration of the relative intensities of the corresponding bands of the two components. Since no trace of a band at λ 6600 Å. is found in the spectrum of *b* (although *a* has a band at this wave length which is 2.5 times as intense as the 6425 Å. band of *b*), one would not expect *b* to have a band at λ 4300 Å. due entirely to an impurity of *a*, because the 4275 Å. band of *a* is not of much greater intensity than is the 4525 Å. band of *b*.

To obtain these absorption spectra curves, an accurate method was used which is far more sensitive to changes of light intensity than is the eye or photographic plate. It is evident that certain bands reported by WILLSTÄTTER and STOLL are not present. In the case of each component, these missing bands would be located in the spectral regions where the most intense bands of the other component are found. These data indicate that WILLSTÄTTER'S components were not pure, and that his separation of them was not complete. The series of seven absorption bands of components *a* and *b* in ether solution show certain similarities in relative intensities. The weaker bands of *b*, however, are not so well separated as are those of *a*. The general structures of the two spectra are the same, while those of WILLSTÄTTER and STOLL'S spectra are dissimilar in some respects.

TSWETT (11), who used the method of preferential adsorption on calcium carbonate to separate the chlorophyll components, reported the absorption spectra of these components, α and β, as observed visually with a microscope equipped with a spectral ocular. Although the color of TSWETT'S component *a* in ether solution was described as a pure beautiful blue, his preparations probably contained some component *b*, carotene, or xanthophyll, because his solutions of greatest thickness showed absorption at λ 4700 Å. appearing as a shadow before the terminal absorption region. TSWETT'S *b* evidently contained some *a* as an impurity, since its absorption spectrum had a band at λ 6100–6150 Å., where *a* absorbs strongly. In the spectrum of *b*, TSWETT did not find bands at λ 6600 Å. where *a* absorbs or at λ 5025 Å. where *b* absorbs. DHÉRÉ and ROGOWSKI (1) prepared the chlorophyll components by TSWETT'S adsorption method, and found that they had the same visible absorption spectra as those given by WILLSTÄTTER. They describe component *a* as pure blue.

GHOSH and SEN-GUPTA (2) studied the absorption spectra of chlorophyll components *a* and *b* prepared by STOLL, in acetone solution. They measured absorption coefficients with a König-Marten spectro-photometer. In so far as the spectrum is concerned their work was not very comprehensive, since they made only fifteen measurements from λ 4350 to 6900 Å.

Recently, WINTERSTEIN and STEIN (14) have reported the purification of both components *a* and *b* by the adsorption method alone. Their method and results for chlorophyll *a* are similar to those described in this paper, although their solvents and adsorptive agent were different. These workers also claim that chlorophyll *b* prepared by WILLSTÄTTER and STOLL'S method contains 15-20% *a*, and their method of removal of this impurity is that of repeated adsorption. WINTERSTEIN and STEIN studied the absorption spectra of chlorophylls *a* and *b* in ether solution by photographic and visual methods. They did not report the 4100 Å. band of component *a*, but found two bands at λ 6070 and 6230 Å., while the writer found only one band in this region, at λ 6125 Å. They report the 6630 Å. band of pure *a* in ether solution to be more intense than the 4320 Å. band. The reverse was found by the writer. The wave lengths of their absorption maxima differ from the values of table I by 5–215 Å. The smaller of these differences are undoubtedly due to errors of their methods of measurement. In the case of chlorophyll *b*, however, an absorption band is reported at λ 6140 Å. by WINTERSTEIN and STEIN (as well as by TSWETT). At this wave length the writer found a minimum of absorption for component *b* and a maximum for component *a* at λ 6125 Å. For this reason the *b* of WINTERSTEIN and STEIN probably contained a small amount of *a* as an impurity. They do not consider the possibility of a third "component *c*."

The methods of absorption spectra measurements employed by these workers were not sufficiently accurate and sensitive to find such small differences as can be detected with the photoelectric method. If the yellow pigments are completely removed by a technique such as WILLSTÄTTER'S, chlorophyll *a* may be isolated in a pure state by a modification of TSWETT'S adsorption method. Component *b*, however, has not been isolated completely from *a* by TSWETT'S method.

In the case of chlorophyll *a*, the pure blue color in ether solution

indicates its complete isolation from *b*. The absence of the 4525 Å. band is the chief reason for this blue color. Small amounts of *a* can-

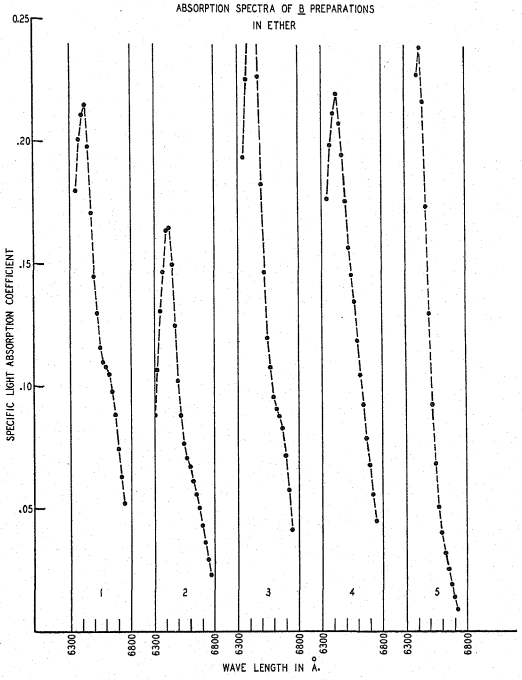

FIG. 2

not be detected in chlorophyll *b* preparations by color observations alone because their ether solutions are green. The best test of purity is an examination of certain small regions of the absorption spectrum

in the ranges of λ 4200–4800 Å. and λ 6300–6700 Å. for components *a* and *b* respectively. In these regions the absorption differences of the two components are greatest. Measurements should be taken at intervals of 25 Å. In figure 2 is a group of these test curves in which the specific light absorption coefficients are plotted as the ordinates. The first four curves (1–4) show the light absorption of *b* preparations from fresh nettle leaves (*Laportea canadensis*). The concentrations of these preparations in ether were only approximately known. Curve 1 is of extract group 1, consisting of extracts 1–4 (step VII 1). Curve 2 is of extract group 2, consisting of extracts 5–10 (step VII 1). In both of these preparations considerable *a* is present, as indicated by the change of slope in the absorption curves at approximately λ 6600 Å., where an absorption maximum of *a* occurs. The *b* of the third extract group, consisting of extracts 11–18, was purified by fractional precipitation (step VII 3, 4). Curve 3 presents the absorption of the fraction which is contaminated with *a* and penetrates the talc; curve 4, which presents the absorption of the precipitated fraction, does not have a change of slope at λ 6600 Å. At λ 6525 Å., a very slight change of slope indicates that probably a trace of *a* remained in this preparation. The amount of material available did not permit a further fractional precipitation of *b*. These curves (3 and 4) show that *b* is more easily precipitated than *a*, which tends to penetrate the talc.

Curve 5 is of *b* from extract group 3, consisting of extracts 11 to 18 (step VII 1). This preparation of *b* was made from fresh barley leaves and contained no *a*. In contrast to curves 1–3, there is no sharp break in the slope on the red side of the 6425 Å. band in the region of λ 6600 Å.

Quantitative analytical method for components *a* and *b*

A quantitative spectro-photoelectric method for analysis of mixtures of *a* and *b* has previously been described in detail (15). Values of α were accurately determined for pure *a* and *b* from the data presented in figure 3. From a measurement of $\log \frac{I_o}{I_x}$ at λ 4400 Å. (α has the same value for *a* and *b* at this wave length), the total chlorophyll ($a+b$) concentration is calculated. The ratio of *a* to *b* is then calcu-

lated from a measurement of α at λ 4100 or 4270 Å., by use of the equations of figure 3. In table II are the results of analyses of mixtures of *a* and *b*. From the experimental measurements of α for known mixtures, as presented in figure 3, the percentage composi-

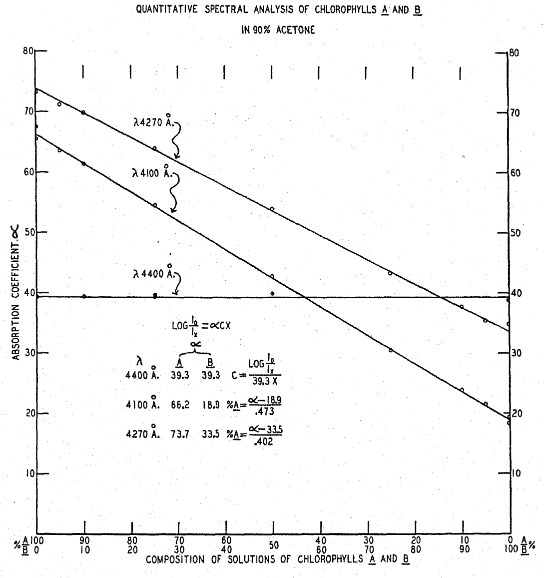

FIG. 3

tions of these mixtures were calculated. The values for the two wave lengths were averaged and the greatest deviation of this average from the true composition is 1.0%. Two unknown mixtures of *a* and *b*, in which neither the total chlorophyll $(a+b)$ concentration nor the ratio of *a* to *b* was known, were analyzed by this method. The compositions, determined by measurements at λ 4100 and 4270 Å., agree

within 1.5%. It is therefore possible by this method to determine the percentage composition of unknown mixtures of components *a* and *b*, with an accuracy of 1.0% or better.

TABLE II

SPECTRAL ANALYSIS OF CHLOROPHYLLS A AND B IN 90% ACETONE

$$\log \frac{I_0}{I_x} = \alpha C X$$

	α		COMPOSITION IN TERMS OF A										
			KNOWNS A+B							UNKNOWNS A+B		"C" FRACTION	
WAVE LENGTH	A	B	5%	10%	25%	50%	75%	90%	95%	1	2	10 MONTHS	24 MONTHS
4100 Å.	66.2	18.9	5.50	10.5	24.3	50.3	75.3	89.9	94.2	65.3	63.5	56.3	25.2
4270 Å.	73.7	33.5	4.73	10.4	24.1	50.7	75.6	90.3	93.8	66.7	65.0	40.1	23.6
AVERAGE % COMPOSITION			5.11	10.45	24.2	50.5	75.45	90.1	94.0	66.0	64.25	48.2	24.4

Solution color study

A study was made of the color of chlorophyll solutions having approximately the same color intensity (table III).

The differences between the colors of *a* and *b* are most pronounced in ether, less in ethyl alcohol, and least in carbon bisulphide. The colors of fraction c are intermediate between those of *a* and *b*. Ether and ethyl alcohol solutions of chlorophyll have a brilliant red fluorescence. Carbon bisulphide solutions do not fluoresce visibly. WILLSTÄTTER usually refers to the color of chlorophyll *a* solutions as blue-green or greenish blue, and only once (13, p. 151) does he state that "the concentrated ethereal solution may be called pure blue."

TABLE III

SOLVENT	*a*	*b*	FRACTION C
Ether (anhydrous)....	*Blue*	Yellowish green	Green
Ethyl alcohol (absolute).............	Slightly greenish blue	Yellowish green	More yellowish than in ether
Carbon bisulphide....	Green, almost like fraction c in ether	Almost yellow	Yellowish green like *b* in ethyl alcohol

Pheophytins of *a*, *b*, and fraction c were made by the addition of concentrated ether solutions of chlorophyll to 95% ethyl alcohol, to which were then added a few cc. of 22% hydrochloric acid. After one

hour, the alcohol was extracted with water and the ether solution stood overnight with 22% hydrochloric acid. The pheophytins were transferred to ether by dilution and washed with water. Dilute solutions of the pheophytins were compared (table IV) as to their color, phase, and later phase (color a few minutes after addition of methyl alcoholic potassium hydroxide).

TABLE IV

Solvent	*a*	*b*	Fraction c
Ether solution	Bluish brown	Yellowish brown	Yellowish brown
Phase	Yellow	Pink	Pinkish brown
Later phase	Greenish	Brown	Brown

The properties of the pheophytin of fraction c are somewhat intermediate between those of *a* and *b*. The later phase colors of *a* and fraction c are particularly different. Ether solutions of the free isochlorophyllides of *a* and fraction c are distinctly different in color: that of *a* is violet-blue and that of fraction c is pure green. Aqueous solutions of *a* and fraction c, previously saponified with hot methyl alcoholic potassium hydroxide, are blue and green respectively, and fluoresce brilliantly. When barium chloride is added to such aqueous solutions, the barium salts are precipitated. These barium salts of *a* and fraction c are purple and green respectively.

Tests of purity

1. Basicity test.—The phytol ester group was undamaged in all of these preparations, because 22% hydrochloric acid extracted no color from their ether solutions.

2. Phase test.—The writer has found that the phase test is useless for detection of contamination of *a* in *b*, or vice versa. The color of the phase is subjective and depends to a great extent upon the concentration of chlorophyll. The phase of *a* is yellow and that of *b* is pink, if the proper concentration is used. The phase of fraction c is usually yellow, but that of some preparations is reddish yellow or brown.

3. Presence of yellow pigments test.—In the phase test, after complete saponification of the chlorophyll (several hours) in ether solution with methyl alcoholic potassium hydroxide, ten volumes of water are added; the saponified chlorophyll remains in the aqueous layer and the yellow pigments color the ether layer, if they are present. When this test

is applied to the components prepared by the preceding method, the ether layers are colorless.

4. Cleavage test.—These preparations did not behave in the cleavage test in the same manner as did Willstätter's preparations, according to his descriptions. It required 22% hydrochloric acid to extract all of the color from the ether solutions of the phytochlorins from *a* and fraction c and the phytorhodin from *b*. Only a part of the color was extracted by 4%, 9%, and 12% hydrochloric acid.

Since all of the chlorophyll preparations gave good phase tests, it seems that the chlorophyll was not allomerized and that allomerization was not the cause of the formation of weakly basic products in the cleavage tests. Possibly the preparations were not saponified under the proper conditions.

5. Absorption spectrum test.—A critical examination of the absorption spectrum, measured with the accuracy of those in figure 1, is the best test for contamination of *a* or *b* by each other or another component. This method of measurement is not subjective, and is very accurate and precise.

Source of leaf material

Fresh leaves were the source of chlorophyll. They were ground and extracted as quickly as possible after harvest, often within 15 minutes. For most of the preparations barley has been used, because it is conveniently and rapidly grown and has proved very satisfactory. It contains less of the non-chlorophyll pigments than do the other plants tried, and the preparation is therefore easier. Wisconsin barley, Pedigree 38, age 1 month, height 10 inches, was used.

Components *a* and *b* have both been isolated from barley and from nettle (*Laportea canadensis*) in the pure state. The blue component, *a*, has also been isolated from the leaves of Kentucky bluegrass (*Poa pratensis*) and Australian rye grass (*Lolium perenne*) from the lawns of the University of California campus, from foxtail (*Hordeum jubatum*), thimbleberry (*Rubus*), and spinach. The typical blue and green zones of step VI 4 were formed from chlorophyll derived from each of these sources (table V). In the vacant spaces of the table, the yields were not measured.

The yields from grass are the average of three preparations. No *a* and fraction c were recovered from the methyl alcohol extracts. The total of *a* plus fraction c is 0.8 gm. per kg. of fresh grass. Both *a* and fraction c would have been included under component *a* by Willstätter and their total yield is the same as his yield of *a* given in his example (13, p. 127),

in which he obtained 4.05 gm. of chlorophyll ($a+b$) from 2.5 kg. of fresh nettle leaves. From 8.0 gm. of chlorophyll he obtained 3.7–4.0 gm. of component *a*, or about 0.8 gm. of *a* per kg. of fresh leaves (13, p. 150).

TABLE V

YIELDS OF PURE CHLOROPHYLL COMPONENTS; APPROXIMATE YIELDS (IN GM.) PER KG. OF FRESH LEAVES

Source	*a*	*b*	Fraction c
Barley	0.1300	0.0145	....
Barley (more succulent)	....	0.0070	....
Lawn grass	0.4200	....	0.3800

Time of preparation

Harvesting and grinding of the leaves, acetone extraction, transference of the chlorophyll to petroleum ether, and removal of excess acetone require about 1.25 hours. The methyl alcohol extractions of *b* require 1.25 hours if these extracts are discarded and *a* is the only component purified; 2 additional hours are necessary if these extractions are to be worked up for *b*. The mixture of *a* and fraction c can be precipitated within 2 hours and their separate ether solutions obtained within 2 additional hours. The time elapsed from the harvest of the fresh leaves to the pure ether solution of *a* is thus about 6.50 to 7 hours. More time is required to isolate pure *b*.

Comparison with other methods of component purification

Since the present method is a combination and elaboration of the methods of Willstätter and Stoll and of Tswett, the following discussion will indicate points of difference between the methods, and will present reasons why the contamination of *a* with *b* (or component *c*) was not suspected by previous workers.

According to Willstätter and Stoll, step VI 2 results in a solution of pure component *a*. That this preparation may be further separated into *blue* and green portions is evident from the talc adsorption experiments. In their method, after the *a* is quantitatively precipitated in petroleum ether by washing out the methyl alcohol, talc is added to collect the chlorophyll particles for filtration. If a small part of the "chlorophyll *a*" were not precipitated, but remained in solution, this added talc would adsorb it and thus prevent

its later separation into zones during filtration. Also, *a* is more easily precipitated from petroleum ether than is fraction c, the green portion, so that the more completely the mixture was precipitated the thinner would be the blue zone of *a*, and the color difference would be less easily noticed. In their method for preparation of pure chlorophyll $(a+b)$, the precipitated pigment "is immediately dissolved from the talc upon the suction filter" with ether (13, p. 123). Thus no opportunity is given for observation of zone formation by any chlorophyll that might not have been precipitated and collected in the top black layer. In their separation of *a* and *b*, the final precipitate of *a* on talc is dried as well as possible with suction. As the talc becomes drier the difference in color of the *a* and c zones becomes less obvious, and both layers become gray. From these considerations it is clear that the more complete is the precipitation of chlorophyll *a* and the more carefully the method of WILLSTÄTTER and STOLL is followed in several details, the less chance has the experimenter of observing the separation of the blue and green fractions. If TSWETT had used an ether-petroleum ether mixture instead of carbon bisulphide as a solvent, more efficient separation of the blue from the green layers could have been made, because the color differences of *a*, *b*, and fraction c are much less in carbon bisulphide solution.

The purification of *b* is very wasteful, but results in a pure product. In the method of WILLSTÄTTER and STOLL, *b* is precipitated from 10 cc. of ether with 400–500 cc. of petroleum ether; while the ratio in the writer's method is 13 cc. of ether to 100 cc. of petroleum ether. All of the methyl alcohol washings (VII) are not used, thus the yield of *b* is decreased further. A less wasteful method for the isolation of *b* in a pure state is much needed for further studies in which larger amounts of *b* will be required.

Number of components and sources of error

It is the opinion of the writer that there is at least one component in the chlorophyll complex, other than components *a* and *b*. Chlorophylls *a* and *b* have been more highly purified than before. This third component, *c*, has not been isolated in a pure state from fraction c, which contains component *a* as an impurity. Consideration of the appearance of two chlorophyll fractions, distinctly different in

color and separated from the preparation formerly considered as chlorophyll *a*, immediately suggests a number of questions regarding possible sources of error in the preparative methods:

(1) Have *a* or component *c* been formed as a result of chemical change of WILLSTÄTTER'S *a* during the purification procedure?

(2) Since the color of fraction c is intermediate between those of *a* and *b* in ether solution, is fraction c a mixture of *a* and *b*, and is its appearance due to incomplete extraction of *b* from *a*?

(3) Is component *c* a polymer or associated product of *a* or vice versa?

(4) Do the fractions *a* and c have different colors because of the presence of some other blue or yellow pigment respectively?

To answer these questions, the components were studied from several points of view, both chemical and physical, and the preparative methods were varied to exclude error. These questions will be considered in turn.

(1) The use of fresh leaves avoids decomposition of chlorophyll due to heating or drying processes. None of the solutions or extracts were heated above room temperature at any time. In the plants studied, acids of the sap do not decompose the pigment during grinding and extraction from the leaf meal. This is evident from the fact that pheophytin does not appear in the final products. Pheophytin would be detected very easily by its strong absorption bands at λ 5000 and 5300 Å. for *a* and *b* respectively, which are located where the pure components absorb least. The pure blue and green colors of the component solutions would thus be changed considerably by the brown pheophytin if it were present. Because of the possibility that a rearrangement of *a* might be catalyzed by the tin walls of the press or the enameled wall of the pan during the extraction, the press and pan were not used in certain preparations. The extract was pressed entirely by hand from the meal into Pyrex beakers. These variations did not alter the results. With some extractions no oxalic acid was used in the 85% methyl alcohol and good preparations resulted, but in other such cases allomerization occurred; therefore oxalic acid was used in all further preparations.

Fresh solvents were used throughout many preparations. Properly reclaimed solvents give equally good results. To make certain that no impurity in any of the solvents was responsible, through oxidation or decomposition, for the appearance of the *a* and c zones in talc, a preparation

was made in which only specially purified solvents were used. Following is a description of the purification methods employed and of the tests applied to the solvents.

Methods of purification of solvents

Acetone (c.p.) was distilled over aqueous Na_2CO_3 and Na_2SO_3 solution, through an 18-inch fractionating column b.p. 56.5° C., odor sweet.

Petroleum ether (Oronite), after standing over aqueous $KMnO_4$ overnight, was distilled. sp. gr. 0.656, b.p. 36–75° C., 50% distilled below 50° and 75% below 60° C. Another grade of petroleum ether, with the following distillation temperatures, was very satisfactory: 50% at 33–40° C., 45% at 40–50° C., and 5% at 50–60° C. The low-boiling petroleum ether distilled as follows: 50% at 31–32° C., 40% at 32–33° C., and 10% at 33–34° C.

Methyl alcohol (12 liters) was treated with 60 gm. of zinc dust and 80 cc. of 50% NaOH solution, boiled under a reflux condenser for 0.75 hour, and then distilled through a 5 ft. fractionating column.

Ether (Baker's anhydrous) stood over sodium chips overnight. It was then distilled over fresh sodium and used immediately.

Tests applied to solvents

The $KMnO_4$ and KI tests were made in closed containers in the dark.

Acetone. (a) For aldehydes: Heat 10 cc. of acetone plus 5 cc. of ammoniacal silver nitrate solution (protected from light) for 15 minutes in a water bath at 50° C. If aldehydes are absent, the mixture should not acquire a brown color and no metallic silver should appear.

(b) Add 1 drop of 1/1000 $KMnO_4$ solution to 10 cc. of acetone and keep below 15° C. The pink color should not disappear entirely in 15 minutes.

Methyl alcohol. (a) For aldehydes: Add 1 drop of N/10 $KMnO_4$ to 10 cc. of methyl alcohol. The red color should not disappear within 10 minutes.

Ether. (a) For ethyl peroxide, hydrogen peroxide, or ozone: Shake 10 cc. of ether with 1 cc. of N/10 KI solution. No color should be acquired after standing 1 hour in the dark.

Other tests used on all solvents were the following:

I. For oxidizing agents: A few drops of neutral KI solution are shaken with 10 cc. of the solvent and allowed to stand for 1 hour. A coloration indicates the presence of an oxidizing agent which oxidizes I^- in neutral solution.

Ia. To I, a few drops of dilute H_2SO_4 are added, shaken, and allowed to stand. The H^+ greatly sensitizes I.

II. For peroxide: A few drops of $TiCl_4$ solution added to 10 cc. of the solvent tested produces a yellow or red coloration if peroxides are present.

III. For aldehydes: Add a few drops of Shiff's reagent to 10 cc. of the solvent tested. A red coloration indicates the presence of aldehyde.

The following tests on the solvents were negative:

ACETONE.—a, b, I, II, and III. Ia was negative for 0.50 hour (slight coloration overnight).

PETROLEUM ETHER.—I, II, and III. Ia was negative for 0.50 hour (slight coloration overnight).

METHYL ALCOHOL.—a, I, II, and III. Ia gave a slight coloration in 0.50 hour and much color developed overnight.

ETHER.—a, I, II, and III.

Excellent preparations of *a* and fraction c were made with solvents purified by the methods just described. Some *a* was dissolved in acetone and left 2 days in the dark. After transference to ether it still gave a good yellow phase test and retained its original blue color.

(1) and (2). Ether solutions (0.1 gm./liter) of *a* are *blue* and cannot be called greenish blue or bluish green. Ether solutions of *b* are yellowish green and those of fraction c are green. The apparent colors of green solutions are somewhat subjective and depend considerably upon the comparison solution. Solutions of fraction c might be called bluish green in comparison with those of *b* if certain concentrations are used. Mixtures of *a* and fraction c are definitely bluish green when compared with *b*. If solutions of *a* and *b* having approximately equal color intensities are used to make one of the same shade of green as fraction c, the mixture must be about two-thirds *a* and one-third *b*. That fraction c was a mixture of *a* and *b* after twenty extractions with 85% methyl alcohol seemed very improbable; nevertheless several experiments were performed to prove that this was not the case and that *a* and fraction c were not changed into one another during the alcoholic extractions and subsequent filtrations.

Fraction c from two preparations was dissolved in ether, added to 1 liter of petroleum ether (b.p. 35–55° C.), and extracted twenty times with 85% methyl alcohol and once with 90% methyl alcohol. The pigment remaining in the petroleum ether was filtered on talc (step VI 4) and gave a thick green layer of fraction c and a very thin blue layer of *a* below, amounting to about 5% of the total. This *a* was evidently not separated from component *c* in the first filtration, which was shown by the fact that

when fraction c, prepared by the usual twenty extractions with 85% methyl alcohol, was dissolved in an ether-petroleum ether mixture and filtered, a very thin bluish layer was found at the bottom of the colored talc. When *a* was extracted twenty additional times with 85% methyl alcohol and filtered on talc in the same manner as in the usual preparation, no green layer was found and the blue zone occupied the total colored portion of the talc. The products left after these last extractions had been extracted forty times with 85% methyl alcohol and three times with 90% methyl alcohol. These may be compared to WILLSTÄTTER and STOLL's sixteen extractions with 85% methyl alcohol and three with 90% methyl alcohol, which supposedly completely removed *b*.

WILLSTÄTTER and STOLL (13) did not state whether the compositions of their 85% and 90% methyl alcohol solutions, used for separation of *b* from *a*, were expressed in volume or weight percentages. It has been assumed that volume percentage was used. A preparation was made, however, in which the methyl alcohol solutions were prepared on the weight percentage basis. The methyl alcohol extracts were all darker in color than when the solutions prepared on the volume percentage basis were used. Only sixteen extracts with 85 weight % methyl alcohol were made, as directed by WILLSTÄTTER and STOLL's method. The sixteenth extract was rather dark. The petroleum ether solution was much more dilute than usual after the sixteenth extract. Three extractions with 90 weight % methyl alcohol, which were dark colored, left the petroleum ether solution definitely bluish in color but it was not the pure blue characteristic of pure component *a*. In step VI 4, the layers of *a* and fraction c were not sharply defined in the talc because the ether concentration was too great. The upper portion was greener than the lower portion, however, and the two ether solutions were different in color, that of the top layer being greener than that of the lower. The yield of *a* plus fraction c was less than usual, and the yields of the first crude *b* fractions were correspondingly greater, because they contained more *a*, which appeared in step VII 3. This experiment demonstrates that even when the 85% and 90% methyl alcohol solutions are prepared on the weight basis, the green c fraction is not completely separated from the *a* and the yields are low.

(3) After saponification in the phase test, when water is added to the mixture, the ether becomes colorless and the lower aqueous solution is blue in the case of *a* and green in the case of fraction c. Alkalies often cause polymerization in solution. Since the aqueous layers, formed by additions of water to the phase tests, remain blue with *a* and green with

fraction c, retaining their color differences, and since *a* and fraction c pheophytin solutions, which have been treated with acid, have different colors, it is very improbable that component *c* is a polymer of *a* or vice versa.

(4) Since the ether solution becomes colorless after addition of water to the phase test of fraction c, the green color is not caused by the presence of carotene or xanthophyll as an impurity of *a*. Xanthophyll esters would be hydrolyzed by the alkali, liberating the xanthophyll to the ether layer. The blue color of *a* cannot be due to an anthocyanin impurity, because the anthocyanins are insoluble in ether, benzene, and other solvents in which chlorophyll is soluble (12, p. 46). The green color of fraction c is not due to an anthoxanthin impurity, since the anthoxanthins and flavones dissolve in water and acids to give yellow and red solutions.

Discussion of fraction c

Efforts were made to prepare the green component *c* of fraction c in a pure state by repeated filtration of the ether-petroleum ether solution of fraction c through talc and separation of the blue *a*. The writer was unable by this means to isolate from fraction c a green component whose properties were constant. The green product always exhibited properties of a mixture.

Fraction c was prepared from fresh foxtail (*Hordeum jubatum*) leaves. In step VII the petroleum ether solution was washed forty times with 85% methyl alcohol, and in step VII 3–6 three filtrations through talc were made to separate the last traces of *b* and *a* respectively. The third talc chromatogram had a very thin blue layer of *a*, about 5% as thick as the green layer. The absorption spectrum of an ether solution of this fraction c, after three adsorption purifications, was examined visually with a small spectroscope. It contained two distinct bands of about equal intensity in the red region, such as would be shown by a suitable mixture of *a* and *b*. The same absorption spectrum was obtained from the fraction c of a preparation from lawn grass after three filtrations through talc (step VI 3–6). When the fraction c from the foxtail was one year old, having been carefully dried and kept in a weighing bottle in darkness, its absorption spectrum was measured in a more precise manner with the spectrophotoelectric method described earlier (16). In general shape the absorption curve was intermediate between those of *a* and *b*. The

bands at λ 4525 and 6425 Å. were predominant over those at λ 4275 and 6600 Å. This indicates that *if* fraction c were a mixture of *a* and *b*, *b* was present to the extent of about 75%. Since (1) the last ten of the 85% methyl alcohol washings were made after the second talc filtration and were very light in color, and (2) only about 5% of the total was removed as *a* in the third filtration, it is very improbable that 75% *b* could have been present in the final preparation. This is evidence that fraction c is not simply a mixture of *a* and *b*, but contains a third component *c*.

These preparative data indicate that at least one component other than *a* and *b* exists in the chlorophyll complex. Although it has not been prepared in a pure state, certain of its properties may be deduced from the foregoing data. Its color must resemble that of *b*. With respect to the separation between petroleum ether and methyl alcohol, this third component *c* has properties similar to those of chlorophyll *a;* considering differential adsorption on talc, its properties are similar to those of *b*.

Melting point data are not reliable as criteria of purity for the chlorophyll components. The sintering and melting points vary considerably with the speed of heating and are often very indefinite.

Two preparations of fraction c were analyzed as unknowns. The results are presented in the last two columns of table II. These samples were not fresh but had been dried, powdered, and kept in a weighing bottle in the dark for periods of 10 and 24 months respectively. Both samples were prepared according to the method here presented, the 10-month sample being purified twice by steps VI 3–6 and the 24-month sample three times by steps VI 3–6 plus ten additional washings with 85% methyl alcohol in step VII. The 10-month sample was isolated from barley leaves and the 24-month sample from the leaves of a closely related plant, foxtail.

The analyses were made in the same manner as were those of the unknowns containing *a* and *b*, on the assumption that fraction c contained a mixture of *only a* and *b*. The total concentration was determined by a measurement of $\log \frac{I_0}{I_x}$ at λ 4400 Å. From the determinations of α at λ 4100 and 4270 Å., for *a* and *b*, the percentage of *a* was calculated by use of the equations of figure 3. If only *a* and

b were present, the results from measurements at the two wave lengths should agree within 1.5%. In the case of the 10-month sample, however, the calculated compositions disagree by 16.2%, which is many times the experimental error. This disagreement proves that there is a third substance which does not absorb light to the same extent as either *a* or *b* at one or more of these wave lengths. It is the opinion of the writer that this third substance is a third component, *c*, of chlorophyll. However, there may be more than one component other than *a* and *b*. These data show only that there are more than the two components *a* and *b* in the chlorophyll complex and set no upper limit to the total number. In the case of the 24-month sample the agreement is fairly good, within 1.6%, as though only *a* and *b* were present. This agreement indicates that component *c* may change to *b* on standing. A sample (*a*+fraction c), as obtained in step VI 2–3, was kept in a dry powdered condition for 3 years. It originally gave a chromatogram of two zones, 1 and 2 (pl. X, B). After 3 years its chromatogram consisted of three distinctly different zones, similar to zones 2, 3, and 4 of plate X, A. The upper one was dark green, the middle zone lighter green, and the lower zone greenish blue. Evidently the *a* had somewhat deteriorated, as its color was not pure blue. The respective zone thicknesses were in the ratio 5:12:17. This is further evidence that component *c* may change to *b* on standing. The nature of this change and its optimum conditions are unknown.

Summary

1. An improved method for the isolation of chlorophyll components *a* and *b* has been developed. The methods of WILLSTÄTTER and STOLL and of TSWETT were combined and modified. The final purification processes involve differential adsorption on talc and fractional precipitation from petroleum ether, for components *a* and *b* respectively. The steps of the procedure are presented in detail.

2. The absorption spectra of chlorophylls *a* and *b* were measured accurately from λ 3950–7800 Å. by a photoelectric method. Absorption coefficients at the maxima and minima were determined with an accuracy of 1.0%. The visible spectrum of each component consists of seven bands.

3. Spectral data indicate that the components prepared by WILL-

STÄTTER and STOLL and by TSWETT were not separated completely from one another. The spectral method for detection of impurities is discussed.

4. A comparative study was made of the colors of *a* and *b* in various solvents and of certain chlorophyll derivatives.

5. Different tests of purity are discussed and it is concluded that a critical examination of the absorption spectrum is the most reliable and sensitive test.

6. Sources of leaf material and component yields are presented.

7. The improved preparation procedure is critically compared with previous methods.

8. It is suggested that there is at least one component other than chlorophylls *a* and *b* in the chlorophyll complex. Possible sources of error in the purification procedure are discussed.

9. Attempts to isolate the third "component *c*" were unsuccessful. Its properties are intermediate between those of components *a* and *b*.

10. Quantitative analytical data affirm the existence of a third component *c*.

This research was started at the Laboratory of Plant Nutrition, University of California, in 1929 and continued at the George Herbert Jones Chemical Laboratory, University of Chicago, from 1931 to 1933. The absorption spectra and methods of purification were presented before the Division of Biological Chemistry at the 85th Meeting of the American Chemical Society, Washington, D.C., March, 1933.

The writer wishes to express his appreciation to those who have given him the opportunity for this investigation: in particular to Professor D. R. HOAGLAND, of the University of California, under whose direction the first work in the purification of chlorophyll *a* and the attempted isolation of component *c* were made; to Professor T. D. STEWART for suggestions on solvent purification; to Professor T. R. HOGNESS, of the University of Chicago, for many suggestions of technique in the absorption spectra measurements; to Dr. E. S. MILLER for making a preparation of component *a* from directions written by the writer to demonstrate that another experiment-

er, unaccustomed to the technique, could secure the same results; and lastly, to the Board on National Research Fellowships in the Biological Sciences for making the latter part of this work possible by the grant of a fellowship.

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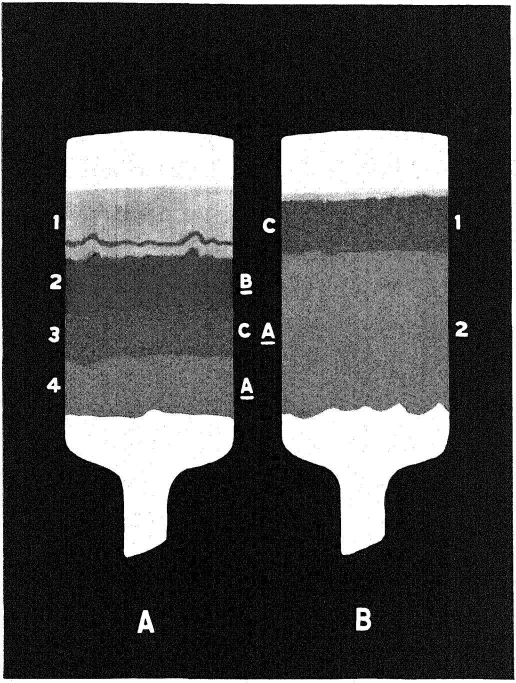


ZSCHEILE on CHLOROPHYLL PURIFICATION

DEVELOPMENT OF SPORE WALLS IN SPHAERO-CARPOS DONNELLII

MARGARET B. SILER

(WITH TWENTY-SIX FIGURES)

Introduction

The persistent tetrads of spores derived from each spore mother cell, which are characteristic of most species and races of *Sphaerocarpos*, were interpreted by BISCHOFF (6) in *S. terrestris* as three-knobbed spores. PETOUNNIKOW (23), realizing that each tetrad is composed of four spores, called these tetraspores because of their resemblance to the tetraspores of the Florideae. LEITGEB (18) and later writers have referred to them as spores persistent in tetrads.

AUSTIN (3) first described *Sphaerocarpos donnellii* from Jacksonville, Florida. According to his description the species has a fragile, deeply lobed coccus [tetrad], each spore of which possesses a large, fragile tubercle. HAYNES (15) and HAYNES and HOWE (16) cite among the specific characters of *S. donnellii*, "spores separating at maturity, ... each while united in the tetrad commonly showing a prominent protuberance about 12 μ high in middle of outer face and after separation exhibiting a coarsely lobed basilar margin." A species whose spores separate early in their development, *S. cristatus*, has been described from California (8, 9, 17).

ALLEN (2) finds that in most of his cultures of *S. donnellii* the spores remain united in tetrads. In a few cultures, however, the spores separate at maturity, somewhat as described for the species by the taxonomic writers. Studies of matings between plants producing sporophytes whose spores remain united at maturity and those in whose sporocarps they separate show that among the descendants of one clone (R27E) the separate-spore tendency is regularly transmitted through the female gametophyte (2). This character follows in inheritance the course of the X-chromosome (1).

Later examination of herbarium material from the type locality for the species (Jacksonville) disclosed the presence of some sporo-

phytes with separate or separable spores and others with persistent tetrads. Plants of *S. donnellii* received from Tallahassee, Florida, in the spring of 1929 had sporophytes with separable or rarely adherent spores; plants from Gainesville, Florida, received the same spring had sporophytes with adherent spore tetrads. Cultures have been made from these two lots of material.

The accomplishment of normal matings (2) between gametophytes from separable and those from non-separable strains suggests that the adherence or non-adherence of spores produced by sporophytes from the respective types of female is due to the presence of one or the other of a pair of allelomorphic genes, which, at least in culture, affect spore wall development. One object of the present study was to determine what, if any, feature in the history of development of the spore wall is responsible for the separation or adherence of the spores.

As early as 1867, PETOUNNIKOW (23) inquired into the structure and development of the "tetraspores" of *S. terrestris*. According to him the spores remain united in fours, surrounded by the walls of the "special mother cells," which become transformed into a reticulate, cuticular envelope. When the septa between spores are apparent, sections of the tetrad wall show three layers: the outermost, yellow at maturity with ridges forming a reticulum, and extending between the spores by means of the septa; the innermost, lining each spore; and a third lying between these two layers. The septa formed by the first layer between the spores do not show a distinct separation into layers belonging respectively to each of the adjoining spores.

LEITGEB (19, 20) agreed with PETOUNNIKOW in interpreting the wall inclosing the tetrad of *S. terrestris* as the transformed "spore mother cell (walls of the special mother cell)." The common outer wall is divided by a network of ridges, caused by folds in the wall membranes, which have "acute spicules" (15) at the intersections. Where this outer wall continues between the spores it is granular and without a middle lamella. Within the outer wall each spore of the tetrad is surrounded by its own exine and cellulose intine. The exine of each spore has a reticulate pattern corresponding to that of the outer wall. The intine has no special structural details. LEIT-

GEB'S description of the formation of these walls will be discussed later. His paper of 1883 (19) was a preliminary account without illustrations. The more extensive and illustrated account of the spore walls of *Sphaerocarpos* (20) to which STRASBURGER refers repeatedly has been unavailable.

STRASBURGER (26) concluded that the spores of *S. terrestris* remain united in tetrads because each tetrad is surrounded by a continuous outer layer of exine. At maturity each spore, inside the common outer exine, is surrounded by a dark brown, finely lamellate layer (the inner exine) and a thick homogeneous intine. The inner exine projects in the ridges of the spore pattern on the outer faces of the spores, but is thin in the septa between spores, being somewhat thicker between the outer margins and between the apices. STRASBURGER distinguished a middle lamella in the septum. The development of the persistent tetrad will later be compared with that found to occur in *S. donnellii*. The correspondence of the outer exine of the tetrads of *S. terrestris* to the outer exine of *Riccia glauca* was pointed out, with the conclusion that this layer is an exine, not a perinium.

LORBEER (21) notes a few features in the development of the tetrads of *S. donnellii*, employing LEITGEB'S terminology (20). Exine and intine originate about each spore. The whole tetrad complex in the meantime becomes surrounded by an increasingly thick gelatinous layer which is apparently built up with the help of the original spore mother cell wall and the nurse cells. In the midst of this layer the undulate structures of the perinium become apparent. The perinium holds the spores fast together.

Riccia is the only other genus of hepatics in which the spore wall development has been studied in any considerable detail (20, 26, 4, 7, 10). STRASBURGER (26) found three layers in the mature spore wall of *Riccia glauca:* an outer and an inner exine, whose folds are coincident except where they are separated by granular material about the basal margin of the spore, and an intine. The division of the spore mother cell is followed by a thickening of the special mother cell wall (that is, the walls surrounding and separating the spores). The thickening proceeds in pillow-shaped areas with depressions between, into which the cytoplasm extends. Upon these thickening

layers each spore deposits a wall of its own. This layer thickens and then a second layer is laid down by the cytoplasm. As the inner exine thickens it becomes yellow, both it and the outer exine becoming cutinized and brown at maturity. Shortly before ripening of the spores the cytoplasm lays down an intine. The thickening layers of the special mother cell wall are finally entirely resorbed. Each spore during its development doubles its diameter.

STRASBURGER did not agree with LEITGEB'S (20) interpretation of the outer exine as a perinium derived from the special mother cell wall by transformation. He held that the earlier development of the outer exine as a tetrad-inclosing layer about the spore mother cell in *Sphaerocarpos terrestris* accounts for the persistence of tetrads in this plant, as contrasted with the separation of the spores of *Riccia glauca*, where the outer exine is laid down around each spore.

BEER (4) re-examined the development of the spore walls of *R. glauca*. In general he confirmed STRASBURGER'S conclusions. He did not commit himself regarding the origin of the first spore wall (outer exine), however, but held the evidence insufficient to show whether this layer is deposited upon the thickened special wall or is developed by transformation from the inner part of the thick special mother cell wall. The second spore wall (inner exine) becomes differentiated into three regions (two being lamellated with a structureless dark-staining layer between). An endospore (intine) is laid down late in the development of the spore. BEER concluded from chemical tests that the thickened special mother cell wall is composed of callose, that the first and second spore walls become cuticularized, and that the endospore is of pectose and cellulose.

BLACK (7) compared the development of spore walls of *Riccia frostii* with that of *R. glauca* as described by BEER. A few points concerning spore wall development are given by CAMPBELL (10) for *R. trichocarpa* and *Fimbriaria californica*, and by HAUPT (14) for *Reboulia hemisphaerica*.

Materials and methods

For this study of the development of spore walls in *Sphaerocarpos donnellii* Aust., matings of various clones were made; the sporophytes were collected and killed in Flemming's medium solution

upon successive days throughout the period of spore development. Sections were cut 7 μ in thickness and stained with Flemming's triple stain, safranin and light green, Haidenhain's haematoxylin and light green, or with methylene blue. The triple stain and the methylene blue gave the best differentiation of wall layers.

The plants used in matings were from ALLEN'S clones and included the following:

♀	♂
31.453 (typ.[1])	×31.745
23.107 (typ.)	×31.773
30.613 (sep.)	×31.1021
29E3 (sep.)	×31.433
25.2409 (sep.)	×31.710
22.22 (sep.)	×31.745
20.22 (sep.)	×31.1021

The typical female 23.107 was one of the offspring of female 21.215 in a mating the sporophytes from which produced spores adhering in tetrads (2); 29E3 and 30.613 were females grown from separate spores from plants from Tallahassee. Females 25.2409, 22.22, 20.22 were descendants of the female clone R27E which always produced sporophytes with separable spores (2). All the males used were descended more or less remotely from a common female ancestor R35G, whose sporophytes always produced tetrads. One male ancestor, 20.45, of all the males was grown from a separate spore produced by a sporophyte derived from the female clone R27E.

Female and male plants to be mated were placed in the same pot on December 13, 1931, and flooded with sterilized water December 27. The fixations which supplied the material for study of the spore walls were made almost daily from January 26 to February 7, and on February 17, 1932, the sporophytes being 30–42 days and 52 days old respectively.

All drawings were made with the aid of a Spencer camera lucida. Figures 1 and 2 were drawn with a 12× planoscopic ocular and a Spencer 4 mm. objective. All other figures were drawn with a 12× planoscopic ocular and a Spencer 1.5 mm. objective.

[1]Typ., spores in tetrads; sep., spores separating.

Observations

Spore mother cells

The fully developed sporophyte of *Sphaerocarpos donnellii* resembles closely that of *S. terrestris* (*S. michelii*) which is often figured (**13**, fig. 214; **22**, fig. 51). The simplicity of the capsule with its single-layered wall (fig. 20), the narrow, short seta, and the bulbous absorbing foot have been abundantly commented upon (**10**, **11**, **22**, **24**).

In the sporophytes here under consideration the central sporogenous tissue of the capsule was separated into rounded cells as early as 19 days after fertilization. These free cells are suspended in a liquid medium in which they continue to develop and grow as the capsule enlarges. During this period of growth a differentiation takes place. Some of the free cells remain comparatively small, green, and packed with starch; others continue to enlarge and gradually lose their chlorophyll and most of their starch content. The former are nutritive cells,[2] the latter spore mother cells.

In either typical or separable races the cytoplasm of a spore mother cell having a diameter of 45 μ is dense at the periphery; in the center it is traversed by radially elongated vacuoles. As the mother cell enlarges the peripheral cytoplasm also becomes vacuolate. The fewer and fewer starch grains are located in the corners of the meshes of the network of cytoplasm.

The mother cell wall at this time is approximately 0.5–0.6 μ in thickness, staining faintly with light green and remaining colorless after the use of methylene blue or of the triple stain. As the mother cell enlarges, a thin dark layer appears on the inner surface of this colorless wall. Within this in turn there develops a third layer which gradually becomes stainable with orange G. This third layer acquires a thickness of about 1 μ in typical spore mother cells of 70–80 μ diameter, and of 1.2 μ in those of the sporophytes from clone

[2] Apparently some nutritive cells enlarge more rapidly and for a longer time than others, for in a single capsule small, several-celled as well as large one- and two-celled nutritive bodies may be seen (fig. 20). As late as 32 days after fertilization, undivided or just divided nutritive cells only slightly smaller than the spore mother cells have been seen. A mitotic spindle was observed in one of these. The spore mother cells in the capsule in which this dividing nucleus of a nutritive cell was found have large resting nuclei and are not ready to divide; each of the smaller nutritive bodies is composed of several cells.

30.613, a separable female. This innermost layer and the thin, more darkly staining layer are usually easy to distinguish. The unstained outer layer can be seen definitely only when a section of a mother cell slips and comes to rest on some other section. In figure 24, a photograph of a spore mother cell, the outer layer of the mother cell wall, transparent to the eye, shows a definite boundary. At this stage, therefore, the mother cell wall consists of three layers. These may still be seen around the young tetrads shown in figures 4, 5, and 11. The three-layered wall breaks away from the protoplast as a unit when the mother cell is plasmolyzed.

The nucleus enlarges as the mother cell grows. In a spore mother cell with a diameter of 45 μ there is a nucleolus, staining very deeply with safranin or haematoxylin, whose apparently rough outline is due to the concentration about it of strands of the chromatin-linin network. The rest of this network is distributed throughout the nuclear cavity. In a mother cell 60 μ in diameter the chromatin-linin network is more sharply defined. The nucleus at this stage and also at somewhat later stages is uniformly excentric in position within the mother cell (fig. 24). In mother cells of a diameter of about 65 μ, in a capsule of a sporophyte from the female clone 30.613, an interesting configuration appears. A large nucleolus containing a clear vacuole lies at one side of the nucleus; a smaller red-staining body lies beside the nucleolus. A number of very small red-staining bodies are seen on the slender strands of the chromatin-linin network which lie about the nucleolus. In mother cells of a diameter of about 80 μ the nucleus is in diakinesis and centrally located. The bivalent chromosomes occupy one side of the nuclear cavity. Figures at this stage are similar in both types of mother cells.

Just before their division the spore mother cells have a very characteristic appearance. In triple-stained preparations the vacuoles show as radially elongated areas in the gray cytoplasm. A few starch grains, pale blue with red centers, are still present. These contrast sharply with the dark blue starch grains in plastids in the nutritive cells and in the cells of the capsule wall. A translucent zone of cytoplasm lies just inside the cell wall. The mother cell stains deeply and rapidly with iron-alum haematoxylin at this stage and destains slowly, while the nutritive cells and capsule wall destain rapidly.

Thus far no distinction has appeared between spore mother cells

that are to give rise respectively to separable and to adherent spores, unless it be the somewhat thicker inner wall layer in the former.

Division into spores

Lorbeer (21) found in his sporophytes of *S. donnellii* that division of the mother cells occurred 42 days after flooding of the parent plants. In my material division occurred from 30 to 34 days after flooding. However, as Lorbeer found, within a given capsule the condition is fairly constant for all mother cells. Young tetrads occur in material fixed as early as 30 days after fertilization (sporophytes of females 29E3 and 30.613 of the separable races), while undivided mother cells are found in some capsules of either type fixed 34 days after flooding. The time of occurrence of the reduction divisions undoubtedly varies greatly with growing conditions.

My preparations show only a few of the stages in meiosis. The nuclear appearance in spore mother cells of various sizes up to the time of division has been briefly outlined in connection with the description of mother cells. Diakinesis was observed in a 33-day separable and in a 34-day typical capsule. The bivalent chromosomes lie close together in most nuclei that I have observed, probably before actual diakinesis. In some nuclei the chromosomes are spread out so that the bivalent structure may be observed, and as many as seven pairs have been counted in one such nucleus. These chromosomes are granular and somewhat larger than those seen later on the spindle. In these same capsules occur several spindles of the heterotypic division, two doubtful cases of the binucleate stage, and in one capsule a single tetrad.

In the 34-day capsule from typical female 31.453, in which most nuclei are in early diakinesis, a spindle of the heterotypic division was seen. Five chromosome pairs are in metaphase; the other three pairs are not present. In the 33-day capsule from separable female 30.613, an anaphase of the heterotypic division was seen. Three separated pairs of chromosomes in one section and four pairs in the next succeeding section account for the seven somatic chromosomes of the haploid complement, but the X-Y pair is lacking. Lorbeer (21) found that the X-Y pair separate before the others and pass to the poles first. This may account for their absence in both the

anaphase and metaphase figures just described. In the anaphase at least one or two of the chromosomes passing to the poles appear double in structure.

No cell plate was seen after the first division. In the two binucleate mother cells observed no division of the cell had occurred. After the homoeotypic division, the mother cell is divided by cell plates (presumably) into four protoplasts, which lie within the mother cell wall (figs. 11, 21). Fibrous cytoplasm with strands perpendicular to the separating plasma membranes is a characteristic feature of newly formed spore tetrads in the separable race (fig. 11), and of the (usually rare) dyads in the typical race. In the dyads this fibrous cytoplasm occupies a broad spindle-shaped area between the two nuclei. In young tetrads of the typical race the fibrous cytoplasm is not very noticeable. A young spore has a large nuclear cavity in which the chromatic material is aggregated toward one side (figs. 11, 21).

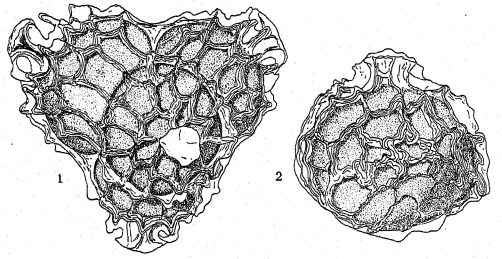
The development of the tetrads or separate spores from the spore protoplasts within the mother cell wall may best be considered separately for each type. The development of the typical tetrad (fig. 1) has been traced in the sporophytes from the mating 31.453×31.745, supplemented by those from the mating 23.107×31.1021. Similarly, the history of the separate spores (fig. 2) has been followed rather fully in the sporophytes of 30.613×31.773, and careful comparison has been made with development in the sporophytes of the other matings of separable females (30.613 and 31.453 were the only females which produced enough sporophytes to allow daily fixation during the critical periods).

Development of walls of typical tetrad

After the division of the spore mother cell the four spore protoplasts lie within the mother cell wall, usually in such a position that an approximately median section of the spherical tetrad shows three spores as about equal sectors of a circle (fig. 21). No middle lamella is laid down between the spores, but very early there may be observed a deposition of material between the outer, slightly rounded angles of the protoplasts and the mother cell wall, like a very blunt wedge. Patches of this same material appear on the inner face of the mother

cell wall against the outer faces of the spores (fig. 3). This is the beginning of what will here be called the "special wall," the term used by GATES (12) in describing pollen-tetrad wall formation in *Lathraea*. It is equivalent to LEITGEB'S (19) "special mother cell wall" and to STRASBURGER'S (26) "thickened mother cell wall," but is a layer separate from the mother cell wall proper.

The special wall material is probably pectic in nature, since it stains deeply with methylene blue, in striking contrast, when it



FIGS. 1, 2.—Fig. 1, typical tetrad of *S. donnellii* showing ridges and characteristic protuberance on middle of outer face of each spore; fig. 2, lateral view of separable spore showing small dorsal protuberance and ridges more pronounced on dorsal (outer) face than on ventral. ×368.

first appears, to the almost unstained mother cell wall (fig. 3) whose thin dark median layer seems to be its outer limit. In some cases all three layers of the spore mother cell wall are distinguishable (figs. 4, 5). The special wall stains with orange G; therefore, in triple-stained preparations the mother cell wall can be distinguished only because it has a discernible inner boundary. Treated with light green, the special wall is bright green, and the mother cell wall has a dull green inner layer and a distinct yellow outer layer.

The cushion-like patches of the special wall become thicker, reaching a thickness of 5 or 6 μ in the middle of each patch. Between these masses there remain depressions, arranged in the pattern of the future wall markings. Each spore protoplast follows the contour of the special wall on its dorsal (outer) face, extending to the tip of

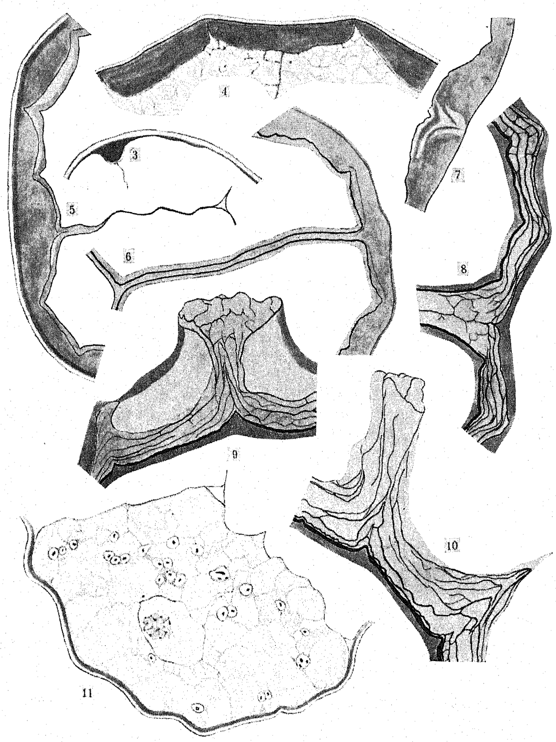
each indentation (fig. 4). The protoplasts are tightly pressed together at their outer angles and there is as yet no wall between their inner faces (fig. 4). Viewed as a flat expanse, the inner surface of the special wall would present a network of furrows, while the dorsal faces of the spore protoplasts would project in a network of ridges, fitting the special wall as a cast fits its mold. The special wall is actually a thick layer deposited by and incasing the tetrad of spore protoplasts which as yet have no complete walls of their own.

Soon, however, each spore protoplast deposits a thin layer completely around itself. As this is the first wall laid down around each spore of the typical tetrad, it will be spoken of as the "first spore wall." This layer when newly deposited stains red-violet with the triple stain, and on the dorsal face of each spore shows distinctly in contrast to the orange-staining special wall, whose contour it follows and to whose inner face it adheres tightly.

The walls along the inner faces (the planes of contact of the spores) now form the first septa between the spores. They lie close together at first, but almost immediately after their formation (fig. 5) a small amount of special wall material appears between them at the outer margins of adjacent spores. Almost simultaneously with the deposition of the first spore wall, the inner part of the special wall (which continues to grow thicker over the outer faces of the spores) shows differentiation into a less dense layer that follows the contours of the new first spore wall, adheres closely to it, and tends to break away from the rest of the special wall (fig. 5). This inner layer of the special wall is continuous with the special wall material of the septa between the inner faces of the spores, suggesting the origin of this latter material by an extension or flowing in of part of the less dense layer. The special wall material finally forms a thin layer between the first spore walls of adjoining spores (fig. 6).

The first spore wall increases in thickness by further deposition. It stains very darkly with methylene blue and is probably of pectic nature. After reaching its maximum thickness it is stained by safranin in triple-stained preparations.

The outer part of the special wall is easily separated from the inner thin special wall layer which immediately surrounds the tetrad. Sections of tetrads fixed at this stage sometimes lose the outer part



FIGS. 3–11.—Figs. 3–10, development of typical tetrad: fig. 3, first appearance of special wall as irregular deposit inside spore mother cell wall on dorsal face of a spore and between margins of two adjoining spores; fig. 4, adjoining margins of two spores showing 3-layered mother cell wall and indented special wall surrounding tetrad, cytoplasm extending to tips of depressions; fig. 5, wall between two adjacent spores and part of their dorsal walls, showing 3-layered mother cell wall, thick special wall over dorsal faces with first lamella and less dense layer just outside first spore wall, and a small amount of special wall material between first spore walls at outer margins; fig. 6, margins of adjoining spores showing differentiation of first lamella outside first spore wall, special wall material completely separating first spore walls on ventral faces, and newly deposited intine; fig. 7, portion of thick special wall on dorsal face of spore after deposition of first spore wall, showing localization of stainable material accompanying differentiation of the lamellae which are beginning development of dorsal protuberance; fig. 8, margins of adjoining nearly mature spores showing intine complete, heavy first spore wall, lamellate layer with four or five lamellae, and undifferentiated special wall material to outside, the mother cell wall being no longer distinguishable; fig. 9, dorsal prominence of nearly mature spore with wall layers same as in fig. 8 except that lamellae have developed outward at angle to build up the prominence; fig. 10, mature dorsal protuberance and prominent dorsal ridge of spore of typical tetrad showing no undifferentiated special wall left and mother cell wall disappeared; first spore wall is heavy and intine thick. Fig. 11, spore of newly formed separable tetrad showing spore mother cell wall that incloses the tetrad, starch grains in cytoplasm, and nucleus with excentrically placed chromatic material. $\times 936$.

of the special wall, then consisting of the tetrad and inner part of the special wall only. The tetrad with the inner part of the special wall may fall out of the sections, leaving only the outer part of the special wall on the slide. STRASBURGER (26) found a similar condition in *S. terrestris*.

At the outer limit of the special wall layer which has become differentiated next to the first spore wall a dark layer appears, as though stainable material had there condensed; this is the first lamella of the lamellate zone which will eventually be differentiated from the special wall. The first lamella parallels the contours of the first spore wall across the dorsal face of each spore, and turns inward between adjoining spores. It does not develop farther inward than the margins of the spores. This lamella is violet in triple-stained preparations.

The thick special wall around the tetrad continues to differentiate a succession of lamellae, apparently by localized processes of condensation outside the first lamella. That the lamellate structures belong strictly to the spores rather than to the tetrad as a whole is shown by sections through the margins of adjoining spores (fig. 8). No one lamella continues unbroken across the gap between spores. Each turns inward around the spore to which it belongs and either ends bluntly or adheres to another lamella. Consequently the ridges of adjacent spores in a tetrad (fig. 1) are contiguous but not continuous.

The lamellate system is not composed of five or six unconnected sheets of material, one inside the other, but is a coherent structure (fig. 8). Although certain of the lamellae stand out distinctly, close examination shows that these, if not adherent to one another, are interconnected by smaller thin lamellae. The lamellae lie within a matrix of lightly stainable material which includes the first less dense layer immediately outside the first spore wall.

At the time of differentiation of the first lamella, in the thicker region of the special wall on the mid-dorsal face of each spore are found the beginnings of the prominent dorsal protuberances and ridges of the mature tetrad. In sections through this region in preparations stained with methylene blue (fig. 7), alternate layers of less and more deeply stained material are seen to lie at various angles to

the first spore wall, instead of parallel to it. The denser of these layers are the first of the outward-projecting lamellae which build up the prominence on the dorsal side of each spore, and correspond to about the inner two lamellae of the protuberance in figure 9. The dorsal prominence is built higher by successive lamellae as the differentiation of the lamellate layer progresses (figs. 9, 10).

The ridges on the dorsal face of each spore become higher with the addition outward of each new lamella. As the tetrad matures, gradual drying out of the lamellate layer probably serves also to accentuate the ridges. The undifferentiated portion of the special wall becomes more and more reduced as successive lamellae are formed. Finally the faint remains of the mother cell wall (or of the special wall) are seen around the tetrad, extending from one ridge to the next. Where the lamellate layer dips in slightly between spores this outermost vestige is easily detected, stretching straight across from spore to spore. In most cases it is undoubtedly lost as the tetrad dries out at maturity, but in some mature tetrads it is still recognizable.

The special wall material between spores condenses irregularly and forms a cementing layer. This is thinnest between the middle portions of the inner faces and thickest between the apices and between the outer margins of adjacent spores (fig. 23). A fibrous structure is evident, especially between the spore apices (fig. 22). Occasionally in mature tetrads there is no condensed special wall between the spore apices (at the center of the tetrad), but an empty space (fig. 23). In such tetrads, however, the fibrous condensed material between the inner faces of the spores is regularly present just as in tetrads with material at the apices. Between the inner faces of adjacent spores the condensation has taken place in relation to each spore; in consequence, a thin, comparatively even sheet of condensed material marks a median boundary in each septum between two spores. In sections this sheet appears as a line traversing the middle of each septum (fig. 23).

An inner layer, the intine or endospore, is deposited by each protoplast during differentiation of the lamellate layer (fig. 6). It fills in the depressions on the inner face of the first spore wall, and has a smooth inner surface. It attains a thickness of about 6 μ.

The walls of a mature adherent tetrad then consist of:

A. Two layers completely surrounding each spore:

1. A thick intine whose inner surface next the spore protoplast is smooth and whose outer surface is ridged.
2. The dark-staining first spore wall which fits the intine like an outer shell.

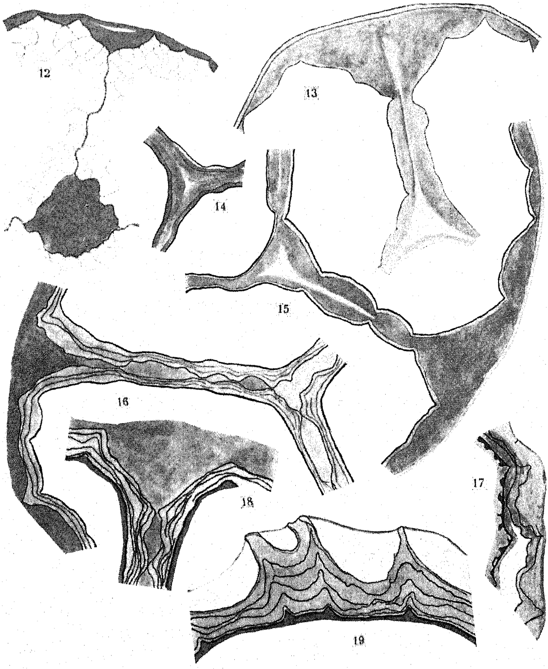
B. One or two layers surrounding the tetrad:

1. The lamellate layer, which is only a thin, irregularly condensed region between the spores but is well developed over the dorsal faces, forming virtually a thick envelope for the tetrad, marked by prominent ridges and by a conspicuous tubercle in the middle of the dorsal face of each spore.
2. Remains of the mother cell wall, if present.

Development of walls of separable spores

In young tetrads destined to become separated, the deposition of special wall material first becomes apparent, as in the typical tetrads, between the outer margins of the spore protoplasts, wedging them apart (fig. 11). This material is distinct from the mother cell wall which incloses the four spores. Along the inner faces of the spores the cytoplasm is fibrous, a condition which might be correlated with the secretion of special wall material by these regions, for special wall material is next deposited between the spores at their apices, in the center of the tetrad (fig. 12). At the same time the special wall masses on the dorsal faces of the spores thicken locally (fig. 12) just as in the typical race, retaining a network of depressions, the forerunners of the spore markings. The deposits between the spore margins increase greatly, becoming large wedges between them and connecting with the special wall material that is being deposited along the inner faces (fig. 13).

The special wall between spores arises as a layer external to the plasma membrane of each inner face. The material between the spore apices spreads out to contribute to this part of the special wall. Depressions of lesser magnitude than those on the dorsal faces are formed in the special wall between the spores (fig. 13), into which depressions the spore protoplasts extend. The indentations of



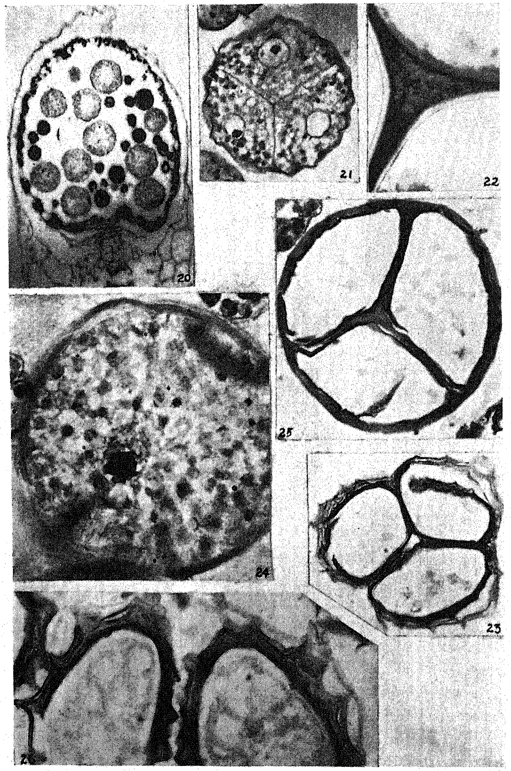
FIGS. 12–19.—Development of separable spores: fig. 12, formation of special wall in cushion-like masses inside mother cell wall and between apices of spores; fig. 13, margins of adjoining separable spores showing special wall between inner faces of adjacent spores with separating clear space extending into the bulge between spore margins, mother cell wall, and deposition of first spore wall as granular material; fig. 14, apices of three separable spores showing differentiation of first lamella in special wall just outside first spore wall; fig. 15, differentiation of first lamella in a tetrad about which the mother cell wall may still be distinguished; fig. 16, portions of two spores showing about three lamellae differentiated from special wall, and dark first spore wall; fig. 17, section of dorsal wall of spore showing from outside in: spore mother cell wall, undifferentiated special wall, two lamellae, first spore wall with irregular deposits of material on inner face, and intine; fig. 18, margins of two spores showing large wedge of special wall between spore margins, about five differentiated lamellae, the heavy, comparatively smooth first spore wall, and intine; fig. 19, section of dorsal face wall of mature spore showing small protuberance built up by lamellate layer outside the first spore wall and intine. This is part of wall of spore shown to right in fig. 26. ×936.

adjacent spores are opposite each other, the protoplast projections thus coming close together at these points.

While the inner special wall is being laid down between the spores it is definitely double. A clear space, dividing it, extends between the two separate deposits almost over the whole of the inner faces (fig. 13). A broader space between the spore apices is also noticeable. Staining reactions show that this is an open space, although probably exaggerated by fixation. Orange G, light green, methylene blue, and ruthenium red all stain the pectic special wall material and leave unstained this clear region, which corresponds in position to a middle lamella. A series of successively older spores show that this space cannot be interpreted as a split progressing from the center outward. The space, at first extending almost to the mother cell wall (fig. 13), becomes limited more and more to the center between spore apices (figs. 14, 15, 25), and finally disappears as the undifferentiated special wall material becomes uniformly distributed between the spores (fig. 16).

During and after the formation of the special wall, the young tetrads destined to become separated are easily distinguishable from those which will remain permanently united in tetrads. In the latter the special wall is developed as a tetrad-inclosing layer, appearing later between the first spore walls of adjacent spores as thin septa. In the separable tetrad the special wall completely surrounds each spore (fig. 25) even before the first spore wall is laid down. It is especially thick between the outer margins of adjacent spores. The mother cell wall, outside the special wall, surrounds either type of tetrad.

The special wall increases in thickness as the tetrad grows, but does not become so thick around separable tetrads as around typical ones. Within the special wall which surrounds each spore the protoplast deposits a layer corresponding with the first spore wall of typical tetrads (fig. 13). Because of its correspondence it will here likewise be called the first spore wall, even though the special wall is actually the first wall layer completely inclosing each spore. The first spore wall follows the contour of the special wall, and is therefore irregularly ridged on the inner faces of the spores and more regularly ridged on the outer faces. This new layer is at first thin, growing thicker,



FIGS. 20–26.—Figs. 20–23, typical race: fig. 20, longitudinal section of capsule containing young tetrads and nutritive bodies, cells of capsule wall containing many dark-staining starch grains. ×72; fig. 21, young tetrad from center of capsule shown in fig. 20. ×312; fig. 22, apices of three spores showing irregular condensation of special wall material, each spore with its own intine and first spore wall. ×880; fig. 23, nearly mature tetrad showing intine, first spore wall, and lamellate layer; nature of septa and junction of spore margins plainly shown. ×312. Figs. 24–26, separable race: fig. 24, spore mother cell showing three layers of wall and excentric nucleus. ×880; fig. 25, differentiation of first lamella in special wall outside first spore wall in young separable tetrad; note broad septa in comparison with fig. 9. ×468; fig. 26, mature spores still lying in tetrad, held together only by mother cell wall stretching from ridge to ridge and from spore to spore. ×468.

probably by deposition from the protoplast, as is suggested by the granular appearance of its inner surface (figs. 13, 25). Figure 17 shows a later stage at which the first spore wall has still an irregular inner surface.

The thickening of the first spore wall is accompanied by a differentiation of the special wall, similar to that which occurs in a typical strain. In a separable race, however, the lightly staining inner layer of the special wall and the condensing lamella surround each spore completely (figs. 14, 15, 25). Figure 15 shows the spore mother cell wall persisting, but all sections do not show it. Preparations stained in methylene blue are most favorable for observing the origin of the lamellae. The special wall where it is in contact with the first spore wall seems to become less dense. At the outer boundary of this less dense zone a condensation of stainable material occurs to form the first lamella. Outside the first lamella another pale (less dense) area may be observed.

The tetrad has been enlarging during the period of wall formation. Its growth has served to straighten out the indentations in the special wall and the first spore wall of the ventral (inner) faces (*cf.* figs. 15 and 16). The latter retain none of the ridges that were laid down in the depressions of the special wall. As the spores grow larger and older, the rounded portions of the first spore wall between the ridges on the dorsal face are also in considerable measure flattened out (*cf.* figs. 15 and 19, figs. 25 and 26).

The special wall on the ventral spore faces shows evidence of lamination earlier than does that on the dorsal faces. In section it has a stringy appearance. While only the first lamella is being differentiated about the dorsal side of each spore, several lamellae may be observed in the ventral faces of the special wall. The ridges of the spore-wall pattern are not marked at first in these lamellae, but as the lamellae become more definite the ridges become more apparent (fig. 16).

The bulge of special wall separating the margins of the dorsal faces of the spores is thickest where three spores abut. There is no marked thickening of the special wall over the mid-dorsal face of each spore as in the typical tetrads; neither is the differentiation of a large dorsal prominence on each spore evident.

After the appearance of the first lamella, more lamellae are differentiated from the special wall completely around each spore (figs. 16, 18). This occurs presumably in the same manner as the differentiation of the first lamella, that is, by alternate condensation and dilution or other modification of special wall material. The lamellae are not separate sheets; they adhere in places or are interconnected by thinner incomplete lamellae. The less stainable material of the special wall, as in the typical tetrad, forms a matrix inclosing the lamellae.

When about three or four lamellae are visible in the special wall around each spore, the intine becomes apparent. As in typical races it fills the hollow ridges of the first spore wall and has a smooth inner surface. The number of lamellae present on the outer face when the intine first appears seems to vary from as few as two (fig. 17) to four or five (fig. 18).

The lamellae follow the contour of the first spore wall, repeating each ridge and broad area between, thus carrying outward the spore-wall pattern. The ridges become a little higher in each new lamella. On the dorsal face of each spore the ridges become higher than on the ventral faces. With an increase in the number of lamellae the mid-dorsal ridges become more prominent than the others, and build up a much lower tubercle (fig. 19) than is characteristic of the typical tetrad. Outside the lamellae may be seen an ever-decreasing layer of undifferentiated special wall around the tetrad and between the spores.

The lamellae of the walls on the ventral faces acquire ridges. These are about as frequent and are situated at approximately the same locations as the original indentations of the special wall and the ridges of the newly deposited first spore wall (*cf.* figs. 15 and 26). The ridges of the first spore wall become obliterated on the ventral faces as the spores grow and the already formed wall layers stretch to cover the increased volume. The first lamella preserves a slight impression of the first pattern and this becomes accentuated by lamellae differentiating later, until a pattern of ridges less prominent than those on the dorsal face is built up on the ventral walls.

As the lamellate layer is differentiated, the thick bulge between spore margins (figs. 15, 16) becomes poorer in stainable material. As the spores round up, their edges are pulled farther apart. Finally,

when the lamellate layer is complete, there remains only a faint vestige of special wall or spore mother cell wall holding the spores in the tetrad arrangement (fig. 26). There is no way of ascertaining which layer this last inclosing wall represents; it is most probably the mother cell wall, for if part of the special wall becomes lamellate, all of it might be expected to be similarly changed. The last-persisting tetrad-inclosing wall is usually lost as the capsule and spore walls become dry at maturity. The spores are then free separate spores. Sometimes, however, the mother cell wall still holds mature spores together in tetrads. Mechanical pressure such as is exerted upon tetrads being mounted on a slide is sufficient to break this outer wall and to free the spores.

The drying of the ventral wall layers is also instrumental in separating the spores. In immature tetrads (fig. 16) the tips of ridges of opposed ventral walls appear to be adherent. In more nearly mature spores (fig. 26) the ridges no longer touch one another, and the vestige of spore mother cell wall is the only structure holding the spores together. The height of these ridges and the depth of the depressions between have increased, and the tips of the ridges have been pulled apart.

The layers in the wall of a mature separable spore (fig. 26) are therefore: (1) The intine, with smooth inner surface and ridged outer face. (2) The first spore wall, a thick, dark-staining layer, granular on its inner surface, smoother on its outer, and fitting like a shell over the intine, its ridges forming a basis (on the dorsal face at least) for the spore markings. (3) The lamellate layer, composed of five or six lamellae in a less stainable homogeneous matrix, in which from innermost to outermost lamella the ridges of the first spore wall are repeated with increasing prominence. The lamellae show greater individuality with fewer cross-connections than do those in the corresponding layer of the typical tetrad.

Discussion

Special wall

The terminology encountered in descriptions of dividing spore mother cells is somewhat confusing because of its origin in studies made before the recognition of the true nature of cells. The cell to early workers was the wall; hence the thick gelatinous wall surround-

ing and inclosing the four parts of a divided pollen mother cell were special mother cells (25). After the true nature of cells was better known, the term special mother cell was transferred to the newly formed spore protoplasts themselves, and the gelatinous wall was called the special mother cell wall, as in the writings of STRASBURGER. LEITGEB (19) used the term special mother cell for either the spore protoplasts or the gelatinous walls surrounding them. PETOUNNIKOW (23), using the old terminology, designated the walls as the special mother cells. As late as 1906, BEER (4) speaks of the spore protoplasts as special mother cells, and of the walls between them as primary and secondary special mother cell walls (equivalent to the terms middle lamella and special wall as used in the present paper).

BENSON (5) has pointed out the absurdity of retaining the unnecessary and erroneous term "special mother cell." The gelatinous wall may still, I think, be called a special wall, since this combination of words is easily identified in older and also in more recent descriptions of pollen and spore walls with structures similar in development.

TYPICAL TETRADS

Comparison of the present description of the development of the wall layers of the persistent spore tetrad in *Sphaerocarpos donnellii* with previous descriptions of the wall development in *S. terrestris* immediately presents some points of difference.

In *S. donnellii* the spore mother cell divides while surrounded by only the comparatively thin mother cell wall, and young tetrads are found inclosed only by this wall. Later the thin mother cell wall of uniform thickness can usually be seen outside the thickening masses of the special wall, which is apparently a new layer deposited by the spore protoplasts. The plane of demarcation between adjacent spores is now formed only by the thin plasma membranes of the ventral faces of the protoplasts. The septa, that is, the wall layers between spores, in a typical tetrad are not formed until after the special wall is well developed around the whole tetrad. Lightly stained preparations of this stage at which the special wall surrounds the tetrad give the impression of being undivided thick-walled mother cells unless the preparation is examined critically.

Both LEITGEB and STRASBURGER reported that in *S. terrestris* the indented special mother cell wall is formed around the mother cell protoplast before the mother cell divides to form the spores. STRASBURGER'S (26) observations seem to have been made largely upon mounts of whole tetrads. In a young tetrad like that shown in figure 20 the presence of four spores would probably not be detected in a whole mount, especially after clearing with glycerin. Whether the spore mother cell of *S. terrestris* divides before or after the special wall appears cannot be stated without re-investigation of that form.

According to LEITGEB (19) no middle lamella is laid down between the spores in *S. terrestris*. STRASBURGER saw a middle lamella in each septum between spores of a mature tetrad of the same species. In *S. donnellii* no middle lamella has been distinguished.

There is evidently a differentiation in *S. terrestris* similar to that which has here been described as occurring in the special wall of *S. donnellii* immediately after the beginning of the deposition of the first spore wall. In the latter species this is plainly a differentiation of the inner portion of the special wall on the outer faces of the spores, immediately following the appearance of the first spore wall. In *S. terrestris* both LEITGEB and STRASBURGER observed this differentiation before the separation of the spores by visible septa. They reported that this innermost layer of the special wall is differentiated around the mother cell. STRASBURGER interpreted it as a wall layer deposited upon the special wall by the mother cell protoplast; therefore he called it the outer exine. The evidence was his observation that the ridges of this inner layer do not reach to the tips of the indentations of the special mother cell wall. He also stated that if a layer were differentiated from the special mother cell wall with its papillate projections, it would not be a coherent layer but would be composed of separate pieces of material. LEITGEB (19) had previously reported that a thin layer and a thicker peripheral layer are differentiated in the special mother cell wall, the inner layer being the forerunner of the perinium which surrounds the mature tetrad.

Observations on *S. donnellii* agree with LEITGEB'S interpretation of the layer in question, namely, that the first lamella and the pale layer inside it are differentiated within the special wall. STRASBURGER'S

first argument may be answered by the fact that in *S. donnellii* the inner layer of the special wall adheres more tightly to the first spore wall than to the outer undifferentiated portion of the special wall (fig. 5). His second objection would not hold in this species. Here either the special wall continues to grow in thickness or the material is always being redistributed, for as each lamella is differentiated the tops of its ridges extend out to the mother cell wall, just as did the depressions in the special wall when first laid down. The lamellae and layers inside them are continuous, and are not composed of separate pieces divided by the original furrows or indentations of the special wall, as STRASBURGER inferred would be the case.

The late appearance of special wall material between the spores in *S. donnellii*, after the deposition of the first spore wall, may be partially correlated with LEITGEB'S observations in *S. terrestris* (19) that the daughter nuclei move apart and the septa between spores are laid down, after which each spore deposits a membrane of its own. STRASBURGER says that the wall separating the spores becomes continuous with the outer exine, after which the inner exine is laid down around each spore. STRASBURGER'S inner exine, when first laid down, and LEITGEB'S membrane around each spore, correspond to what has here in *S. donnellii* been called the first spore wall.

LEITGEB says that the inner lamellate portion (perinium) of the special mother cell wall increases in thickness as the outer layer loses its massiveness; the outer layer is finally reduced to a peripheral lamella that eventually disappears. This process in *S. terrestris* corresponds almost exactly to the differentiation of the lamellate layer from the special wall in *S. donnellii*. The spore mother cell wall which is left around the tetrad is equivalent to LEITGEB'S "peripheral lamella."

STRASBURGER'S account of the maturation of the wall layers is different. According to him the inner exine is the layer that becomes lamellate, and the outer exine, which forms a complete layer around the outside of the mature tetrad, thickens to only a small extent. The special mother cell wall with its thickening masses is lost as the other wall layers grow. STRASBURGER'S outer exine corresponds to the outermost layer of LEITGEB'S perinium, while his inner exine includes the rest of the perinium plus the exine around each spore.

The terms exine and perinium have been avoided in the present paper. What they signify is largely an academic question. That the special wall is deposited originally by the spore protoplasts is evident in *S. donnellii*. When it first appears it might be called an exine. The first spore wall is an exine also, corresponding closely to the exine of many other spores. But the lamellate layer, developing as it seems to from the special wall, can hardly be called by the same name as the exines of the spores of *Riccia glauca* or of pollen grains, which, according to descriptions, involve little if any of the special wall.

Separable spores

The development of the separable spores must be compared with that of the separate spores of other hepatics as well as with that of the spores of typical tetrads in *S. donnellii*. In the few separate-spored hepatics for which any details of spore-wall formation have been given (see Introduction), the thick special wall between and around young spores has been noted. This is the case in *Riccia glauca*, the history of whose spore walls has been most fully studied. Beer (4) states that the secondary special mother cell wall of callose is deposited about each spore within the mother cell membrane and within the primary special mother cell walls between spores (the middle lamellae). No such middle lamellae were observed in the separable tetrads of *Sphaerocarpos donnellii*, although the special wall had the same distribution as described by Beer.

Leitgeb (20), to judge from Strasburger's statements, evidently interpreted the outermost layer of the spore wall of *Riccia glauca* as a perinium derived from an inner layer of the special mother cell wall. If this is the case, then the outer layer of the spore wall of *R. glauca* is comparable in origin with the lamellate layer of the separable spores of *S. donnellii*. Strasburger concluded that this outermost layer in *R. glauca* is an outer exine, deposited by the spore protoplast inside the special mother cell wall and therefore not a perinium. If this interpretation is correct, the outer exine of *R. glauca* is not comparable with the lamellate layer of separable spores of *S. donnellii*. Beer did not attempt to settle the question of the origin of this outer layer in *R. glauca* because of the insufficiency of his evidence.

The inner exine of *Riccia* in its mature 3-layered condition (4) differs from the single-layered first spore wall in *S. donnellii.* The spores of both forms have comparable intines.

The young tetrads of separable spores of *S. donnellii* resemble the young tetrads of *Riccia glauca* (4, 20, 26), *R. frostii* (7), *R. trichocarpa*, *Fimbriaria californica* (10), and *Reboulia hemisphaerica* (14) in the distribution of special wall material, that is, in the feature which apparently is associated with the separable spore character. However, the layers present in the mature wall and their development are features characteristic of *S. donnellii,* exactly comparable only with the corresponding wall layers and their development in the typical tetrad.

Basis of separate or adherent spore character

A comparison of the mature spore walls of the separable and non-separable spored races of *S. donnellii* shows the only conspicuous difference to be in the distribution of the lamellate layer. In the separable tetrad this layer is of comparatively uniform thickness around each spore. In the adherent tetrad it is thick over the dorsal faces, with a prominent tubercle developed on each spore, but thin and irregularly condensed between spores so that it holds them together. The developmental history of the spore wall shows that the location of the lamellate layer of the mature wall is determined by the distribution of the special wall material of the young tetrad. If the special wall is deposited only on the dorsal face of each young spore protoplast, the spores will remain united in tetrads at maturity; if each protoplast becomes completely surrounded by the special wall, it will develop into a separable spore.

Spore wall pattern

A comparison of the number and distribution of the indentations in the special walls of tetrads of *S. donnellii* and *S. terrestris,* and of separate spores of *S. donnellii* and *Riccia glauca,* indicates a correspondence of these indentations with the ridges of the specific tetrad- or spore-wall markings which are diagnostic of mature spores or tetrads.

Leitgeb (19) ascribed the configuration of the wall layers deposited inside the special mother cell wall, and of the layers formed

from this special wall in *S. terrestris*, to the primary vacuolate structure of the protoplasm and to the swelling pressure of the persistent mother cell wall.

Certain advantages may conceivably be ascribed to an indented special wall. On the outer face of each spore with such a special wall the protoplast retains connection with the mother cell wall until the first spore wall is laid down. This condition may allow a more rapid intake of water and food materials. The first spore wall and the successively formed lamellae in turn each repeat this connection of their ridges with the mother cell wall, that is, with the outer limits of the special wall.

A papillate surface has a greater area than a smooth one; therefore a wall layer deposited over such a papillate surface would be better suited to cover an expanding body than a similar layer deposited upon a smooth surface. A simple mathematical computation demonstrates the advantage of the deposition of the first spore wall upon the series of approximately hemispherical thickening masses of the special wall.

A uniform layer of material deposited on the surface of small hemispheres on the inner face of a large sphere has about twice the surface of the large sphere, and hence can be expanded without increase in area to cover a sphere of twice the volume of the original one and with a diameter of 1.414 ($\sqrt{2}$) times the original one. Measurements of typical tetrads at the time the first spore wall is deposited over the papillate surface of the special wall, and of similar tetrads when mature, show on the average an increase in the distance from apex to outer face of from 41 to 52μ, 1.29 times the original dimensions. The fact that the increase of the spore cavity is as slight as this seems to show that the first spore wall, once deposited, does not need to grow by intussusception to accommodate the growing protoplast. It is deposited originally with a surface area adequate to cover the protoplast of the mature spore, and may as a consequence be a limiting factor in the final size of tetrad or spore.

Summary

1. Spore mother cells of both typical and separable spore races of *Sphaerocarpos donnellii* develop in a similar manner, dividing to form young spore tetrads inclosed by a thin mother cell wall.

2. In a tetrad of a typical race, a gelatinous special wall, composed of hemispherical masses with depressions between, develops about the tetrad and later is extended as a thin layer between the spores. In a separable race the hemispherical masses of special wall develop also over the inner faces of the spores.

3. In both races there is deposited about each spore a darkly staining first spore wall with ridges corresponding to the depressions in the special wall, and a thick intine.

4. The special wall in either case becomes differentiated into a lamellate layer. In the typical tetrad the lamellate layer is thick over the dorsal faces of the spores, where it develops a prominent tubercle, and thin and irregular between spores, holding the tetrad together. The lamellate layer surrounds each spore of the separable tetrad on all faces, with a much less prominent dorsal protuberance.

5. Typical and separable tetrads differ at maturity mainly in the distribution of the lamellate layer. Thus the original distribution of special wall material determines whether the spores will remain united or will become separable as they mature.

6. The indented special wall, and the first spore wall following the contour of the special wall, allow for the expansion undergone by the spore protoplast during the maturation of the spore walls.

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PHLOEM ANATOMY IN TWO SPECIES OF NICOTIANA, WITH NOTES ON THE INTERSPECIFIC GRAFT UNION

A. S. CRAFTS

(WITH PLATES XI, XII AND NINE FIGURES)

Introduction

Recent studies on the movement of organic nutrients in plants (6, 7, 8, 14, 15, 19) indicate the important rôle of the phloem in this process. Numerous researches, however, have failed to develop a thoroughly satisfactory theory to explain the rapid flow of materials. Although the sieve tubes seem fundamentally involved, their exact place in the mechanism is yet to be determined. Even more recent studies (1, 11, 12) indicate that certain insect-borne viruses spread within the plant, after their introduction, through the phloem tissues. Here again the sieve tube apparently takes part in the process of translocation; and, on strictly anatomical grounds, its ability to conduct seems closely related to its ontogeny. In the sugar beet, internal symptoms of curly top are initiated relatively close to mature sieve tubes (11).

BENNETT showed (1) in his ringing experiments that the causal agent in curly top could not pass a ring unless there was a bridge of vascular tissue. In grafts, he found that the curly top agent moved from one member to the other only after vascular continuity had become established. As two species of *Nicotiana* have played an important part in these studies, providing the material for the work on translocation, it seems justifiable to investigate in detail phloem development as related to the formation of the graft union and to conduction across it in these species. The present work describes phloem ontogeny in *Nicotiana glauca* Graham and in *Nicotiana tabacum* L. It indicates the nature of union in the interspecific graft, shows how rapidly the first vascular connections may be formed, and discusses phases of the process that should interest horticulturists in general.

Normal phloem anatomy

The phloem of *N. tabacum* will be described in detail, *N. glauca* being mentioned only when interspecific differences occur. Primary phloem of the stem, produced by differentiation of the procambium strands, consists of sieve tubes, companion cells, and phloem parenchyma. In the stage immediately following division of the sieve-tube mother cell, the young sieve-tube element is an elongated cell with transverse end walls, a heavily staining nucleus, and one or more slime bodies (figs. 1, 5). These bodies are often found in close association with the nucleus but not constantly so.

In the young sieve tube, the nucleus is well defined and contains one or more nucleoli. The slime bodies are homogeneous, ovoid, or spindle-form structures, definite in outline and easily distinguished. Although visible in unstained living cells, they may be more readily observed after staining with water-soluble anilin blue.

In the youngest sieve-tube elements there also occur small spherical particles that stain darker than the bulk of the cytoplasm. These appear to be the primordia of the characteristic sieve-tube plastids. They later become much more prominent.

After treatment with IKI and water blue, the protoplasm of the young sieve tube appears granular in structure, usually with one or more prominent vacuoles. In living tissues these vacuoles accumulate neutral red, and the protoplasm exhibits active streaming. With certain killing treatments the protoplasts of the cells are shrunken, the protoplasm readily pulling away from the side and end walls.

With increasing age the nucleus enlarges and decreases in density of staining; the nucleoli shrink and finally disappear (fig. 2). The slime bodies enlarge, assuming a more complex internal structure (figs. 2, 6). Their form changes, becoming longer, less dense, and more spindle-like. The plastids increase in numbers and size, while the vacuoles unite to occupy the whole central lumen.

As the sieve tube matures the nucleus enlarges further; the contents become roughly granular, then thread-like; the nuclear membrane grows thin and disappears, releasing the thread-like particles into the vacuole, where they disintegrate. The slime bodies, enlarging, assume a complex stranded structure and start to fragment,

usually from one end (figs. 2, 6), leaving the lumen occupied by a mass of thread-like material that tends to adhere to the internal protoplasmic strands. This material later breaks loose and, under the influence of certain killing agents, may be induced to accumulate at one end of the cell, forming the so-called "slime plug" (figs. 3, 7). In the normal living cell these thread-like strands are affected by protoplasmic streaming and thermal agitation to produce the peculiar motion previously described in potato (7). They slowly disintegrate, leaving within the cell an amorphous colloidal suspension, subject to the agents just mentioned and able to produce amorphous slime plugs (fig. 4). In the living tissue this colloidal material finally disappears, leaving no coagulable contents in the sieve tubes (fig. 8).

In the maturing sieve tube the protoplasm stains less densely than in the young element. It assumes a fibrous structure when stained, and takes principally a parietal position as the vacuoles unite. The plastids enlarge, are stained pink or reddish brown with iodine, and assume what appears to be a spherical form.

Meanwhile the sieve plate, which up to this stage has differed from other end walls only by an uneven staining of the middle lamella, begins to thicken; the protoplasmic strands that traverse it may be stained. Neutral red accumulation by living sieve tubes becomes less and less pronounced, while all streaming movements of the protoplasm cease. In the mature element the protoplasm forms a thin parietal layer, with a meshwork of very fine strands traversing the lumen and connecting the sieve plates and side wall pits. Vital stains are not accumulated, and plasmolysis cannot be accomplished even with concentrated salt or sugar solutions.

The small callus cylinders that eventually surround the protoplasmic strands of the sieve plate are initiated as the elements mature. First appearing as small collars located on opposite sides of the plate, they increase in length, uniting at the middle lamella and extending farther and farther into the lumina as the plate thickens. Finally they fuse laterally to form a perforated mass through which the greatly stretched protoplasmic strands extend. The plastids, attaining full size, leave the parietal protoplasm. Many adhere to the the inner meshwork of threads, while others apparently become free within the vacuole, exhibiting rapid Brownian movement.

In the petiole and leaf the phloem remains primary throughout the life of the plant, so that sieve tubes of the type just described may be found in various stages at almost any time. In the stem secondary growth soon displaces the primary tissues, and sieve tubes of a different type are found. Secondary sieve tubes are larger, on an average, than primary ones, being broader, usually somewhat curved in form, and having diagonally or longitudinally arranged sieve plates. The diagonal plates have one or more sieve fields; the longitudinal ones often have a greater number.

The slime bodies common to primary sieve tubes have not been found in secondary ones. Arising from cambium cells, the secondary tubes show also a different nuclear behavior. With the early differentiation of the element, the nucleus enters the prophase stage characteristic of mitosis, one or even two divisions rapidly following. The fully differentiated element contains from two to four resting nuclei, which very soon disintegrate, following essentially the same course as do those of the primary tube. A similar behavior has been noted in gymnosperms by STRASBURGER (20). Plastids are very prominent in secondary sieve tubes; and, accumulating carbohydrate, they remain the only inclusions characteristic of the mature element. The cytoplasm is principally parietal with a few strands traversing the lumen. Like the primary, the secondary tubes also lose their ability to accumulate vital stains and to be plasmolyzed; evidently they, too, become completely permeable with maturity.

In the stem of the growing or blossoming tobacco plant most of the sieve tubes are in the "mature stage" just described. All primary sieve tubes are finally obliterated, and young enlarging elements form only a very small fraction of the active phloem tissues.

In tobacco the protoplasmic strands of the sieve plate are extremely fine, varying from 0.2 to 0.1 μ or less in diameter. Starting with an initial length of 2 to 3 μ, they are stretched to many times this length as definitive callus is laid down. The parietal protoplasmic layer and the internal strands become extremely thin and may be demonstrated only by careful staining.

As the permeability of the maturing sieve tube increases, the elements lose turgor and can no longer maintain their form in competition with surrounding living cells. This condition is evidenced by

the slow pushing in of the side walls and the complete closing of the lumina with obliteration. As the contents are resorbed, nothing remains to mark the position of the tube except the thickened regions in the walls and occasional sieve plates crowded between phloem parenchyma cells.

The companion cells change but little with maturity of the phloem. Starting as narrow cells with dense cytoplasm containing small vacuoles, they increase slightly in size, the vacuoles enlarging somewhat. The protoplasm remains relatively abundant, retaining all the properties it possesses in normal living cells. It shows streaming movements, accumulates vital stains, and reacts normally to plasmolysis. Only at senility of the sieve tube does the companion cell degenerate and collapse, finally becoming obliterated with its sieve-tube element.

Phloem parenchyma consists of elongated cambiform or rectangular cells of approximately the same size as sieve-tube elements. Expanding rapidly as they mature, their vacuoles unite to fill their lumina; the protoplasts become parietal; chloroplasts attain their full development. In their mature form phloem parenchyma elements display all the properties of normal living cells. Upon obliteration of the sieve tubes, the surrounding parenchyma cells expand slightly to occupy the space. Certain phloem parenchyma cells are early filled with crystal sand. These persist through the life of the stem, and their numbers may increase in older plants.

In *N. glauca*, phloem ontogeny follows the same course as in *N. tabacum*, with no characteristic deviations in the various stages that would serve to distinguish the two species. Certain structural variations serve in differentiating the two, however, especially in the phloem of the graft union.

The pitting of the walls is more pronounced in *N. tabacum* than in *N. glauca*, a fact indicated in figures 1–8. In *N. tabacum* all phloem elements average slightly larger than in *N. glauca*, and the vascular rays are noticeably broader. In the xylem the vessels of *N. tabacum* are composed of short, relatively broad elements; those of *N. glauca* are noticeably longer and narrower.

Tobacco graft union

Many publications describe the formation of the graft union in fruit trees and in various other horticultural plants. The present study primarily concerns the establishment of vascular continuity in the union of the two tobacco species mentioned, and other works will be cited only if related to this problem.

WAUGH (21) pointed out years ago that the vascular tissues of stock and scion consist of fully differentiated cells, many of which are dead. To him it was obvious that these could not grow together. More recent works (2, 3, 10, 13, 18) clearly describe the intermingling of callus cells from the stock and scion, the establishment of a cambium layer within this callus tissue, and the development of vascular tissue in continuous sheets across the region of the union external to the woody cylinders of the original members.

The present studies on tobacco included both interspecific and intraspecific grafts of *N. tabacum* and *N. glauca*. The process of union formation was found to differ in several details from that taking place in trees. The interspecific grafts showed no signs of incompatibility and differed in no essential from the intraspecific ones. Unions of various ages were studied and grafts of several types were used. The only factor appreciably affecting the formation of the union was the matching of the scion and stock. The more closely the cambiums of these were matched, the more smoothly and rapidly was the union formed.

After the stem was cut in the procedure of grafting, there occurred a slight shrinking of pith, phloem, and cortex cells, with some browning of the cut surfaces. In many cases callus formation started within 48 hours. These grafts differed from those studied by SASS (18): callus was produced by division of parenchyma cells in the pith, phloem, xylem, and cortex, as well as by the cambium. Within a few days the entire surfaces of scion and stock were covered by a layer of callus tissue, which often completely filled any intervening spaces. In as few as five days after the graft was made, regenerative processes had started within the callus tissue; and after divisions in series of these cells, sieve tubes (fig. 9) and xylem elements (fig. 10) were simultaneously differentiated, connecting the younger vascular

tissues of stock and scion. Soon the mature xylem elements of stock and scion were filled with wound gum, while mature sieve tubes rapidly declined, becoming blocked with definitive callus, discolored where actually cut, and obliterated within a short time.

The regenerative stimulus seemed to emanate from the ends of young growing vascular elements, and only in continuity with the latter were the new connecting elements differentiated. When the vascular cylinders of stock and scion were well matched, the paths traversed by these connecting elements were short and relatively straight; when matching was poor, the paths were long and devious.

There seems to be no doubt concerning the origin of these connecting strands. In both phloem and xylem the cells are short, nearly isodiametric, and often oddly shaped. Their walls coincide with those of the surrounding callus parenchyma, from which they have apparently been derived by differentiation (figs. 9, 10). The sieve tubes have no slime bodies and resemble secondary sieve tubes of the stem in every way except form, while the xylem elements show the characteristics of secondary tissue.

By the time these connecting vascular strands are differentiated, cell division in the callus between these xylem and phloem elements gives rise to cambial tissue. By lateral extension, the cambiums of the strands unite to form a complete layer; and in cases where matching is close, a smooth sheet of tissue soon starts differentiation. Within two weeks a continuous layer may be formed, uniting the stock and scion. Development of this layer produces within the region of the graft an annual ring, differing in no essential feature from that of the stock or scion.

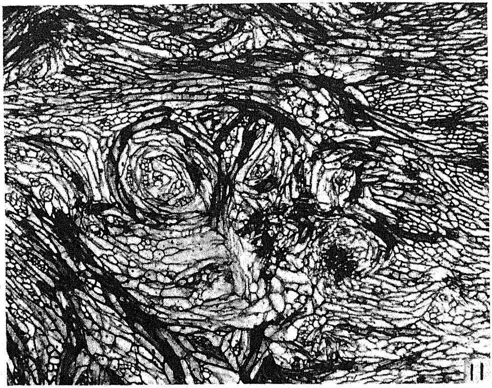
The orientation of the cambium initials produced within the callus is apparently determined by that of the vascular elements between which they lie. When the paths of the vascular elements are straight, the cambium arises as a smooth layer with longitudinally arranged elements of normal form. The vascular tissues, however, occur in strands. When these strands are very long and crooked, the cambium strips formed within them follow the same paths; and lateral connection between them must take place across broad masses of undifferentiated callus cells. The formation of the cambium layer is delayed, and when it is completed the initials lie in odd and varied

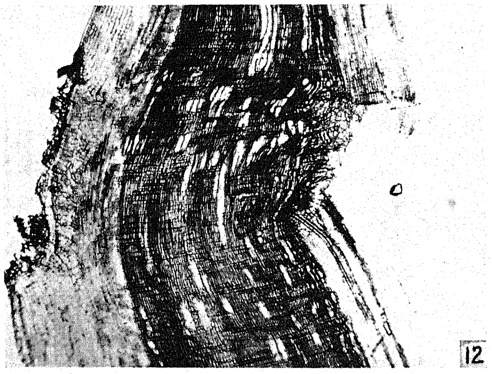
patterns. Vascular tissues differentiated from these initials assume the same pattern, as illustrated by the phloem in figure 11. When the matching of stock and scion is very poor, excessive amounts of this abnormal vascular tissue are formed and the graft union is rough. In those woody species in which there is no incompatibility, the cambium initials may slowly reorient and the graft may, within a year or two, become fairly smooth (2, 18). The strength of such grafts does not seem to be affected, and only when masses of callus or dead vascular tissue interrupt the cambium layer do the grafts remain permanently weak (2, 17, 18). In tobacco the graft may become rapidly smoother. Figure 12 shows the change that has taken place in such a union within a few months.

Detailed studies of the tissues along the line of union in an interspecific graft have indicated several anatomical features characteristic of this region. A particularly smooth union, several months old, was used in the studies illustrated below. It was formed by a long diagonal splice. The vascular elements had been set at a slight angle and the lack of alignment that persisted helped in differentiating the tissues of the two species. Figures 13–16 illustrate this union; they are so arranged that *N. glauca,* the stock, appears on the right and *N. tabacum,* the scion, on the left.

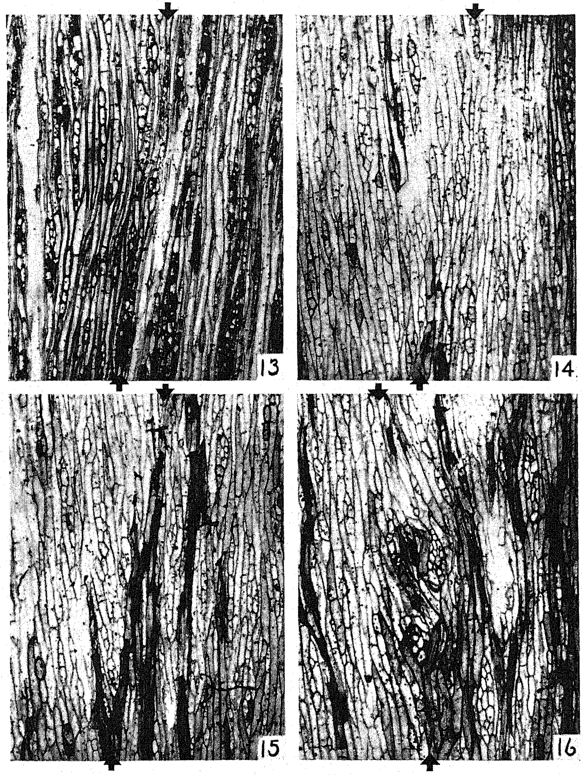
Figure 14 shows the union in the cambium; figure 15, in the phloem; and figure 13, in the xylem. The line of union is denoted by the arrows in the photographs. In the lower part of figure 15, opposite the arrow, a sieve tube may be seen to branch, one arm continuing on the *N. glauca* side and the other entering the *N. tabacum* tissue. Figure 17 is a more detailed illustration of the phloem along the line of union. Although the cells are somewhat misshapen, no irregularities in pitting could be found; and a special technique for illustrating plasmodesma (5) showed no abnormalities in these. The dark structure in the center is a lateral sieve plate, slightly out of focus.

In many unions studied, some abnormal formation of definitive callus occurred in sieve tubes of the stock. This was most pronounced when the line of union was transverse. Although definitive callus formation is characteristic of the old sieve tubes approaching obliteration in all stems, in this special case active sieve tubes right up to the cambium layer showed this condition, which was localized

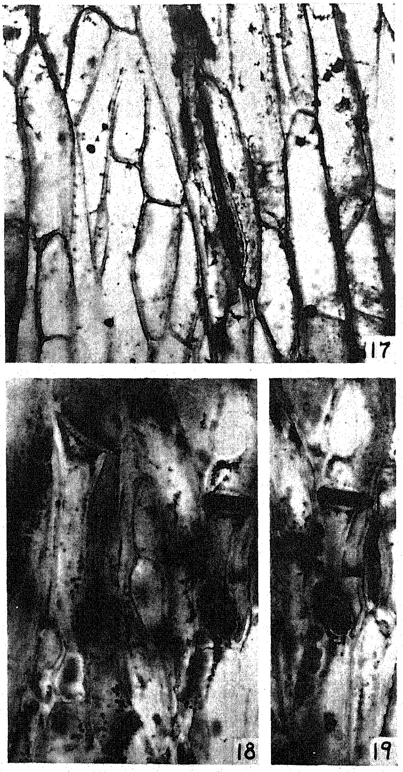




FIGS. 11, 12.—Fig. 11, secondary phloem in longitudinal tangential section from graft union. *N. glauca* on right, *N. tabacum* on left. ×45. Fig. 12, graft union of *N. glauca* (above) and *N. tabacum* (below). Secondary growth has proceeded for several months and radial distortion has been largely overcome. Paths of xylem vessels are still not straight in tangential plane as indicated by open vessel ends shown. Original cut surfaces of bark have been forced to exterior. Phloem elements lie in varied patterns, somewhat as in fig. 11, and sieve plates display excessive definitive callus. ×22.



FIGS. 13–16 (from sliding microtome sections of fresh material stained with iodine and anilin blue).—Fig. 13, longitudinal tangential section of xylem in region of graft union of *N. tabacum* on *N. glauca*. Fig. 14, similar section through cambium. Fig. 15, phloem of the union. Sieve tubes frequently branch. Fig. 16, phloem in a different location, showing "islands" of tissue commonly found along line of union. All ×83.



FIGS. 17–19.—Fig. 17, longitudinal tangential section of phloem on line of graft union. Figs. 18, 19, abnormal callusing of sieve plates of the stock, *N. glauca*, in interspecific graft; two views shown at slightly different foci. Characteristic sieve-tube plastids can readily be seen in fig. 18, even in heavily callused cells. ×430.

within a narrow band adjacent to the line of union. The plastids in these callused cells were full sized and prominent. In senile sieve tubes they decline and disappear. Figures 18 and 19 illustrate the callused sieve plates and numerous plastids in some of these sieve tubes.

In many unions there also occurred small groups or "islands" of cells along the line of junction. These could be found at the time of cambium initiation in new grafts and gave the appearance of root initials. Later adventitious roots were found on several scions, apparently being first formed in the meristematic tissues along the line of union. Figure 16 shows them in the older union previously described (figs. 13–16). Phloem parenchyma cells surrounding these "islands" were usually well filled with starch. The occurrence of root initials accompanied by food storage indicates that the channels of conduction in the phloem were partially clogged. There were no other signs of incompatibility, however, and the grafted plants were perfectly healthy.

Discussion

With the demonstration that the sieve tubes of two *Nicotiana* species behave like those of potato and the cucurbits, namely, that they become completely permeable with maturity, the assumption that this permeable condition is causally related to conduction in the phloem seems strengthened. BENNETT (1) has recorded rates of virus movement approximating those calculated for conduction of foods by the writer (4, 6, 7) and others (9, 15, 16). His ringing experiments with tobacco parallel those of physiologists in demonstrating the relation of phloem continuity to this movement. Other current studies (11, 19) indicate the essential part played by sieve tubes in conduction. Two further indications of the very specialized nature of secondary sieve tubes in tobacco are their multinucleate condition and their differentiation from callus parenchyma cells. Evidently these elements have unique properties peculiar to no other cells of the plant, during certain stages in their ontogeny are adapted to the function of conduction, and are the seat of several very sensitive responses. They should receive careful study by those interested in physiology as well as anatomy, both in healthy and in diseased plants.

The studies just described on the formation of the graft union bring out its relation to conduction. Evidently the scion is practically independent of the stock until the calluses intermingle. Then, in tobacco, vascular elements are soon differentiated and, from the physiological standpoint, the union is formed. Subsequent initiation of a cambium and establishment of normal secondary growth provide more efficient vascular connection and the supporting tissues necessary for prolonged existence of the new plant. Vascular continuity and provision for the required processes of conduction, however, seem to be made before the cambiums are united.

Certain writers (3, 10, 18) give the impression that, in woody species, continuity of the cambium is established first and that the earliest vascular elements are derived from this cambium. SASS describes the process as thus taking place in apple. Only when poor matching was followed by excessive callus formation and delayed cambial bridging did he find the oddly shaped xylem elements characteristic of the first xylem connections in tobacco. KOSTOFF does not mention the exact order of these processes in his grafts. Although he says, "The cambial connection appears first in the lowest region of the callus" (13, p. 570), he later describes and illustrates "abnormal vascular bundles" that correspond closely to the strands differentiated before cambium formation in the unions studied in the present work. BRADFORD and SITTON (2) are somewhat indefinite on this particular detail. In discussing congenial unions (p. 47) they say, "Union occurs first in a zone of parenchymatous tissue, which becomes gradually cut up by tracheids, vessels, and fibers, and finally disappears. . . . . If the fit of stock and scion is particularly close, the zone is very limited in extent and quickly disappears." Here they seem to imply the formation of xylem strands within the callus parenchyma. In describing the union in the bark (p. 52), however, they state, "Sometimes . . . . there is a growth of parenchymatous nature from the cortical regions, establishing a union between stock and scion. Whether this is established or not, new bark is formed as soon as the cambium layers unite." Many of their illustrations show small oddly shaped xylem elements resembling those found in the tobacco graft unions. Continuity of the cambium possibly pre-

cedes differentiation of vascular elements in some species; however, since the vascular tissue that differentiates prior to cambium formation "quickly disappears," as just noted, it may have been overlooked by some investigators.

Regardless of the order in which these various tissues appear, vascular continuity in tobacco is rapidly established in the graft union. As soon as the elements become functional, paths are open for the movement of the curly top virus across the union. Since differentiation of xylem elements and sieve tubes occurs simultaneously, the finding of any mature vascular elements in a tissue bridge is good evidence that both these systems have become functional. There seems no reason to doubt that the ringed stems transmitting virus in BENNETT's studies (1) were bridged by wound callus within which functional phloem had differentiated. Apparently there are no exceptions to the theory that the transmission of curly top virus occurs only in the phloem.

Summary

1. The structure and development of sieve tubes seem causally related to normal conduction in plants, to the movement of certain viruses, and to the formation of the union in grafts and wounds.

2. Stages in the ontogeny of phloem elements in tobacco differ little from those described in potato. Primary sieve tubes contain slime bodies, nuclei, plastids, and the usual cytoplasmic structures. The young elements accumulate neutral red and may be readily plasmolyzed. As they mature, the nuclei and slime bodies disintegrate, vital stains do not accumulate, and the cells can no longer be plasmolyzed.

3. Secondary sieve tubes have no slime bodies. As they leave the cambium, the nuclei may divide one or more times to form as many as four nuclei in the differentiating cell. Later these nuclei disintegrate, plastids develop, and the cells acquire the usual properties of mature sieve-tube elements. Obliteration follows the same course as in potato.

4. Vascular strands differentiated from callus parenchyma connect stock and scion within as few as five days after grafting.

5. Cambium arises within these strands; and by lateral extension a complete layer is soon formed, continuous with the cambiums of the stock and scion.

6. Orientation of the cambium initials depends upon that of the original vascular strands. Where the matching of stock and scion is good, the strands are short and straight, and the cambium approaches in form that of the normal stem. Where matching is poor, the strands are long and crooked, while the cambium initials lie in varied patterns. Grafts of this type are so rough that conduction may be hindered. When no incompatibility exists, they usually become smoother with time.

7. Formation of definitive callus occurs abnormally early along the line of union, especially on the side of the stock. Sieve-tube plastids in these callused cells are normal in form and numbers.

8. No irregularities in pitting of cell walls could be found along the line of union in the *N. glauca-N. tabacum* grafts.

9. Root initials, surrounded by parenchyma cells heavily stored with starch, characterized the line of union in several grafts.

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EXPLANATION OF PLATES XI, XII

PLATE XI

FIG. 1. Young sieve-tube elements of *N. tabacum*, showing slime bodies, nuclei, and vacuoles in cytoplasm. ×455.

FIG. 2. Slightly older sieve-tube element with slime body somewhat fragmented and nucleus expanding prior to disintegration. A mature element appears on left. ×455.

FIG. 3. Sieve plate of *N. tabacum* with slime plug composed of fragments of disintegrated nucleus and slime body. ×455.

FIG. 4. A more mature element showing amorphous disintegration products; plastids conspicuous in last three views. ×455.

FIG. 5. Young sieve-tube element of *N. glauca*, slightly more mature stage than in fig. 1. ×455.

FIG. 6. Fragmenting slime body in *N. glauca.* ×455.

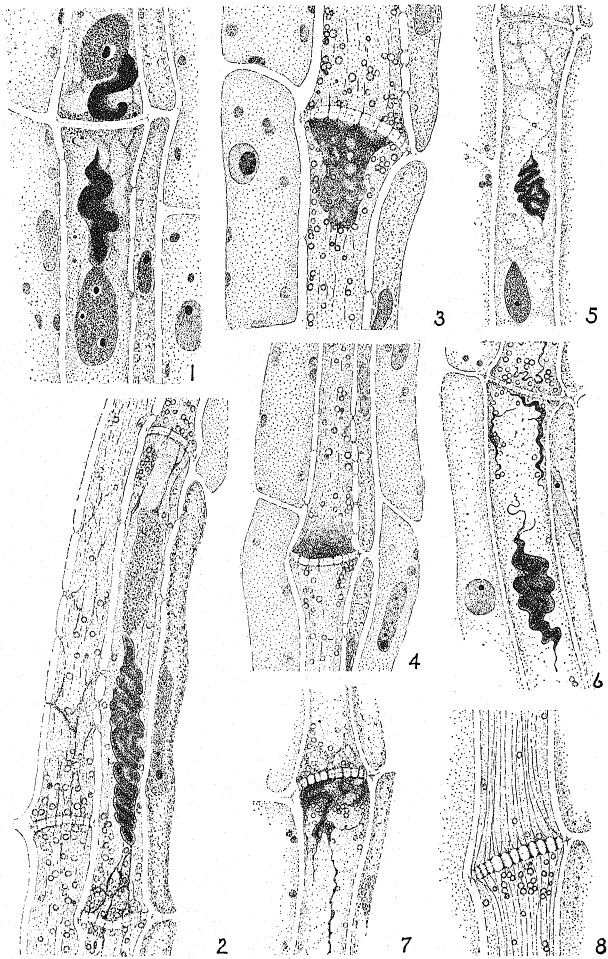
FIG. 7. Sieve plate of *N. glauca* with slime plug composed of distinct fragments of the slime body. ×455.

FIG. 8. Sieve plate of mature element in *N. glauca* in which all disintegration products have left the cells, while the protoplasm shows clearly a parietal layer and an internal stranded condition. ×455.

PLATE XII

FIG. 9. Sieve tube differentiated from callus parenchyma cells within callus tissue of graft. ×410.

FIG. 10. Xylem vessel formed by differentiation of callus parenchyma cells. Shape, size, and matching of these elements indicate their origin from callus cells. Walls between xylem elements contained large pores; true vessels are evidently formed by this process. ×410.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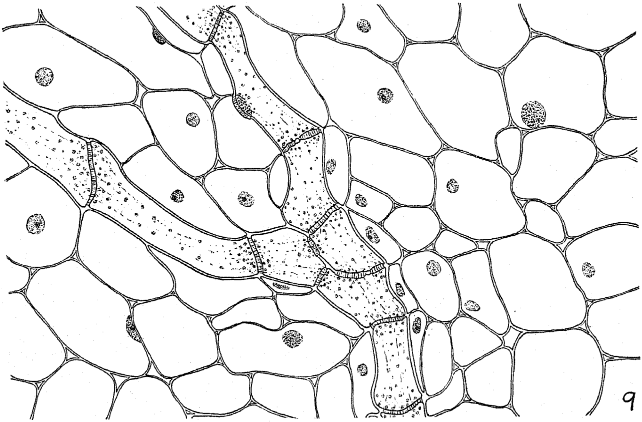

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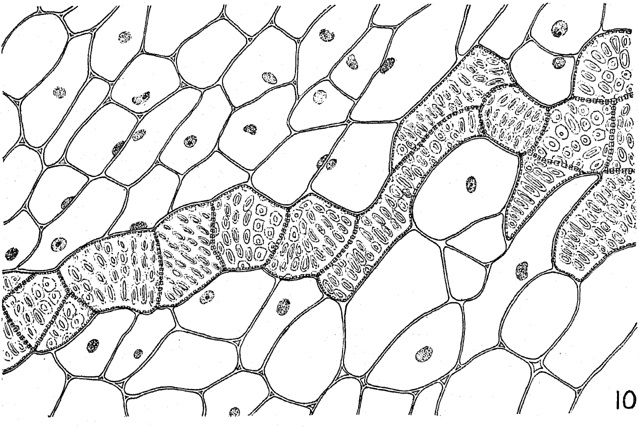

10

CRAFTS on PHLOEM ANATOMY

A CYTOLOGICAL ANALYSIS OF SELF-STERILITY IN TRADESCANTIA

EDGAR ANDERSON AND KARL SAX

(WITH THREE FIGURES)

Introduction

Many species of *Tradescantia* are strongly self-sterile. MOORE (6) reported 83 self-pollinations as completely sterile, and in all the cultures of *Tradescantia* now under cultivation at this institution no self-fertile plants have as yet been discovered. Table I summarizes

TABLE I

SPECIES	NAME AND NO. OF PLANT	NO. OF SELF-POLLINATIONS	NO. OF SWOLLEN CAPSULES	NO. OF SEEDS RIPENED
T. reflexa	Algonquin 52	25	0	0
T. reflexa	Ullin 20	25	0	0
T. reflexa	Dexter 1	25	0	0
T. gigantea	Gigantea 4T	8	0	0
T. montana	Balsam Gap 10	25	0	0
T. bracteata	Portage des Sioux 47	10	3	0

the results of a series of controlled self-pollinations. In only one plant was there any indication of self-fertility, although all the plants used in the experiment had been proved fertile in crosses with other individuals, averaging a little more than three morphologically perfect seeds per pollination. Even in this one instance the plant was not truly self-fertile, since the few seeds which developed were small, shrunken, and practically empty. A cytological analysis of self-sterility was therefore undertaken, since the large nuclei and clear cytoplasm of *Tradescantia* facilitate investigation which in other genera would be difficult, if not impossible.

Investigation

METHODS.—Practically the entire study was based upon aceto-carmine smears of self and cross-pollinated styles. The plants used were grown in pots in the experimental greenhouse. Before *Trades-*

cantia flowers open they are sensitive to handling and bud emasculation is not ordinarily practicable. The flowers open shortly before sunrise (varying somewhat with the temperature and the humidity), and pollen is not ordinarily shed until much later in the morning, particularly in the case of plants kept in the greenhouse. It is therefore possible to emasculate the plants satisfactorily after the flowers have opened. Pollen was used from freshly opened flowers, and as far as possible the work was carried on during sunny weather. It has been found (unpublished data) in *Tradescantia* that during cool cloudy weather the percentage of imperfect pollen is greatly increased. A few of the pollinations were made on plants kept for a day or two in the laboratory, but for the most part plants were pollinated in the greenhouse and the styles brought into the laboratory. When the length of the pollen tube was in doubt the whole style was smeared, but when it was approximately known, the "empty" lower portion of the style was cut away before the smear was made. In a few cases the styles were slit in half with a dissecting needle before they were mounted. The styles were placed on a slide, covered with a drop of aceto-carmine and a cover glass, and then pressed gently but firmly until they were smeared flat. It was found advantageous to heat the smears gently until they were almost up to the boiling point; the preparations were then sealed with wax and were ready for use. The lengths of the pollen tubes were obtained by making camera lucida drawings at bench level and measuring the drawings.

As seen in aceto-carmine smears, the ungerminated pollen grain of *Tradescantia* is comparatively homogeneous, with a dark-staining, sharply defined generative nucleus and a much less well-defined vegetative nucleus (8). The generative nucleus is already a coiled body, many times longer than broad, and becoming progressively longer as germination proceeds. It assumes various positions, although it most characteristically assumes a coiled and twisted U-shape. It is often possible to show that the two ends of the U are unlike, since one is terminated by a short and very definite knob or granule (fig. 1 *C*). In many preparations the nucleus has the appearance of being divided longitudinally by a deep crease, and sometimes an actual fissure is visible nearly the whole length of the nucleus.

The vegetative nucleus is much more hazy and amorphous. There is some evidence of organization and very often an appearance of more or less parallel lines of a deeply staining material. The two nuclei are characteristically closely associated. They may be at either end of the grain or in the middle, but except in rare cases they are either close together or are actually intertwined. The commonest position of the vegetative nucleus is between the arms of the generative nucleus, as shown in figure 1. Germination may take place at one end of the pollen grain, at both ends, or along the side, the first condition being by far the most common. The grain apparently germinates at the point where it touches the stigma. Since the grains are longer than broad they naturally are lodged endwise, among the long papillae of the stigma (fig. 2), and germinate from the proximal end. In the one or two instances where grains were observed to have germinated along the side, it could be seen that they were lying with their long side against the stigmatic papillae. Neither nucleus leaves the grain until the tube has reached a considerable length. Although the vegetative nucleus usually precedes the generative nucleus down the tube, it does not invariably do so. Numerous cases were found in which the generative nucleus was well in advance.

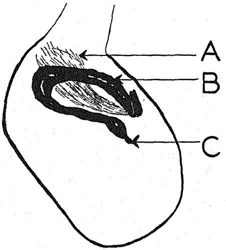

FIG. 1.—Germinating pollen grain, drawn with camera lucida (*A*, vegetative nucleus; *B*, generative nucleus; *C*, granule at one end of nucleus). ×900.

Compatible and incompatible pollinations were made simultaneously on different flowers of the same plant, and were examined at intervals of from 15 minutes to 3 days. The difference between the two types of mating was apparent as soon as 15 minutes after pollination and became increasingly conspicuous thereafter. Germination apparently took place almost immediately in both cases, but the incompatible matings were characterized by slow growing pollen tubes. In the compatible matings, the generative nuclei began to leave the grains and pass down the tubes in about 40 minutes to an hour

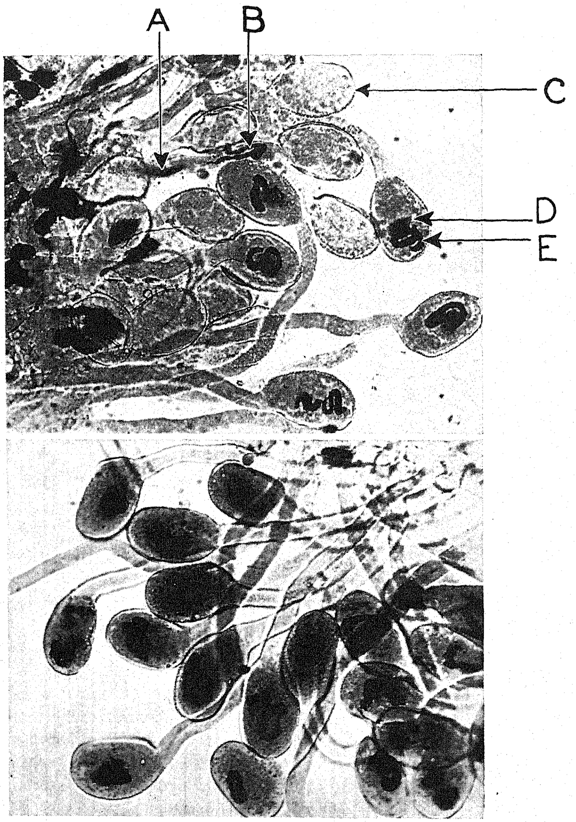

Fig. 2.—Photographs of aceto-carmine smears of pollinated *Tradescantia* styles. Above: cross-fertile (compatible) pollination; below: self-pollination (incompatible). Smears made 5 hours after pollinating (*A*, vegetative nucleus passing down pollen tube; *B*, generative nucleus in same tube; *C*, pollen grain from which nuclei have departed; *D*, vegetative nucleus in pollen grain; *E*, generative nucleus in same grain). Note denser cytoplasm and absence of "empty" pollen grains in the incompatible mating.

while in the case of incompatible pollinations few of the nuclei left the grain within the first 24 hours. The records of a typical experiment are given in detail in table II and photographs of smears are shown in figure 2. Comparative growth curves for compatible and incompatible matings are shown in figure 3. It is difficult to construct really accurate growth curves for two reasons. In the first

TABLE II

COMPARISON OF SMEARS FROM CROSS-FERTILE AND CROSS-STERILE MATINGS; POLLINATIONS MADE IN THE LABORATORY ON POTTED PLANTS OF T. GIGANTEA

TIME	CROSS-FERTILE	CROSS-STERILE
30 min.	7×3 Three-quarters of grains germinated; tubes mostly longer than grain; longest tubes six times length of grain; generative nuclei all in grains	7×2 Only a few grains germinated; tubes mostly shorter than grain; longest tubes scarcely longer than grain; generative nuclei all in grains
1 hour	2×3 Nearly all grains germinated; nuclei mostly in grains, a few in tubes	2×7 A few grains germinated; longest tube four times length of grain; nuclei all in grains
2 hours	2×3 Many nuclei in tubes; several tubes in lower half of style	2×7 Nuclei all in grains; no tubes in style proper
8 hours	7×3 Many tubes half-way down style, a few at base; over half of nuclei in tubes	7×2 No tubes half-way down style, longest barely beyond stigma; nuclei all in grains

place different pollinations on the same plant, made within one or two minutes of each other, may vary 15 minutes in the onset of germination. In the second place there is such great variation between the individual pollen tubes in any compatible cross that it is difficult to arrive at a reliable average. The longer tubes are much more likely to break in the process of making the smear than the shorter ones. Figure 3 shows the range in variation of tube length as well as the average length, but it should be remembered that for the two-hour period the latter figure, in the case of the compatible mating, is little more than an estimate.

One point, however, is clear. The growth curve for the incom-

patible matings is depressed; there is some inhibitory process at work which is progressively slowing down the rate of pollen tube growth. It is apparent within the first few minutes of growth and becomes increasingly conspicuous as growth proceeds. Whether, on the other hand, the compatible tubes grow at a constant or at an accelerating speed cannot be determined from the available data. In the com-

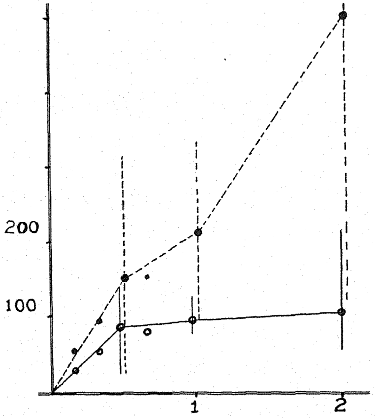

FIG. 3.—Growth of pollen tubes in compatible (broken line) and incompatible (solid line) crosses. Below: time in hours; vertical scale: pollen tube length in 100 μ. The lines connect averages (medians) and the vertical broken lines extend to the extreme values obtained at each interval. The six unconnected points at 10, 20, and 40 minutes are the averages obtained in another set of experiments.

patible crosses pollen tubes were found at the base of the style in four to five hours. The longest tubes found in incompatible crosses of this age were only 150 μ. Since the styles of *Tradescantia* were about 6–7 mm. long, the growth rate of the compatible crosses was about 25 μ per minute and that of the incompatible one about 0.5 μ per minute.

In the case of the incompatible matings the uniform color of the cytoplasm could not be observed to change in smears made at suc-

cessive intervals after pollination. In the compatible matings it became increasingly differentiated (fig. 2) and large vacuoles were often visible. The ends of the pollen tubes assumed different shapes in different experiments. They were sometimes slightly swollen, sometimes of the same diameter as the tube itself, and sometimes pointed. On the whole there seemed to be an association between pointed ends and incompatible matings and between swollen ends and compatible matings, although apparent exceptions were noted. The whole matter, like many others connected with these experiments, is difficult of determination because of the extreme variability which seems to characterize pollen tube behavior.

In many self-sterile species, pollination tests have revealed the presence of cross-sterility between different individuals. In many of these genera it has been possible to group the individuals into a number of intra-sterile, inter-fertile classes, all the members of each class behaving, so far as their compatibilities are concerned, like a single self-sterile individual. (See WILLIAMS and SILOW **11**, p. 344, for a summary of genera and review of literature.) An attempt was accordingly made to see whether it might be possible to diagnose cross-sterility by purely cytological methods. Crosses made at random among *Tradescantia* plants collected from the same locality revealed the presence of many cases of cross-sterility. Cytologically no qualitative nor quantitative difference could be found between these cross-sterile matings and self-pollinations. The rate of pollen tube growth was the same, and the nuclei had the same dark turgid appearance.

In the diploid species and varieties, compatible and incompatible matings were so strikingly different when examined cytologically that it was possible to work out the composition of the intra-sterile, inter-fertile classes by cytological examination alone. As a check on the cytological ratings, a number of matings, recorded as cross-sterile or cross-fertile by cytological examination, were actually made as controlled pollinations. In every case there was complete agreement between the cytological prediction and the actual compatibilities.

Three diploid species were studied intensively, *T. gigantea* Rose, *T. humilis* Rose, and *T. edwardsiana* Tharp. All three gave the same

general results, although they differed in minor points. The division into classes was sharpest in *T. gigantea* (table III), that is, there were no plants whose class relationships were in doubt. In *T. humilis* there was a single exceptional plant (no. 3, table III) which showed an intermediate degree of cross-fertility with all the members of one class, both when used as a male and as a female. In *T. edwardsiana* there was some indication of one intra-sterile class, but

TABLE III

COMPATIBILITIES BETWEEN PLANTS OF SINGLE LOCALITY IN T. GIGANTEA AND T. HUMILIS: −, INCOMPATIBLE MATING; +, COMPATIBLE MATING

T. HUMILIS N.D.D.

♀ \ ♂	11	25	3	18	22	16	5	10	6
11	−	−	±	+	+	+	+	+	+
25	−	−	±	+	+	+	+	+	+
3	±	±	−	+	+	+	+	+	+
18	+	+	+	−	+	+	+	+	+
22	+	+	+	+	−	+	+	+	+
16	+	+	+	+	+	−	+	+	+
5	+	+	+	+	+	+	−	+	+
10	+	+	+	+	+	+	+	−	+
6	+	+	+	+	+	+	+	+	−

T. GIGANTEA

♀ \ ♂	1	4	5	6	14	15	2	7	10	8	9	4u	4w	3
1	−	−	−				+			+			+	
4	−	−	−	−	−	−	+	+	+	+	+	+	+	+
5		−	−	−			+	+	+	+	+	+	+	+
6		−	−	−				+	+	+	+	+	+	+
14			−			−		+		+				
15														
2							−			+			+	
7		+	+	+	+	+	−	−	−	+	+	+	+	+
10			+	+				−	−	+	+	+	+	+
8	+	+	+	+			+	+	+	−	+	+	+	+
9		+	+	+				+	+	+	−	+	+	+
4u		+	+	+				+	+	+	+	−	+	+
4w	+	+	+	+				+	+	+	+	+	−	+
3		+	+	+	+	+	+	+	+	+	+	+	+	−

there were several inconsistencies. It was among these very plants of *T. edwardsiana*, however, that there were found the two individuals with interchanged chromosomes, producing mitotic irregularities which have been reported elsewhere (7). There is also a high percentage of interlocking between non-homologous bivalents in several of the plants of this collection. It is therefore possible that compatibility relationships are disturbed either directly or indirectly by chromosome interchange. If a considerable proportion of the plants in the colony were duplicate for the loci affecting self-sterility, one would expect the same lack of sharply marked classes which characterizes polyploid self-sterile plants (1). It does not seem profitable to discuss possible interpretations of the compatibility rela-

tionships in *T. edwardsiana* at greater length until there are available genetic as well as cytological data.

Very little is known about the number and distribution of intra-sterile, inter-fertile classes in natural populations. East and his co-workers (2, see detailed bibliography) have reported the presence of a great number of such classes in experimental populations of *Nicotiana*. In *Antirrhinum*, Gruber (5) has reported that the number of such classes distributed in nature must be very great. In *Tradescantia* there are apparently an enormous number of such classes in natural populations. In *T. gigantea* seven classes were identified among the 14 plants collected and studied. These plants came from two small areas, situated no more than 300 feet apart, and neither one larger than a table top. In *T. humilis* 9 classes were found among the 12 plants collected in a single field. In *T. edwardsiana*, among the 20 plants studied four belonged to one intra-sterile group. Aside from two other plants whose class relationships were uncertain, there were no other cases of cross-sterility.

Genetical interpretation

While in general the cytological data on self-sterility in *Tradescantia* are in accord with what we might expect on the oppositional factor hypothesis, there are several points of minor disagreement, as will be noted below. It may therefore be profitable, before discussing the genetical interpretation of the results, to consider what kinds of relationships between the pollen tube and the style might possibly obtain in self-sterile plants. The preceding cytological data give clear evidence on several points and effectively rule out certain possibilities. Pollen germination is not affected. The reaction between the pollen tube and the style is apparent almost immediately after germination and seems to be general throughout the style. This agrees with the condition reported for *Nicotiana* (4), but differs from the phenomena observed in *Trifolium* by Silow (9) and in *Hemerocallis* by Stout and Chandler (10). There is also clear evidence in *Tradescantia* that there is a strong inhibitory force at work in incompatible matings. On the basis of these findings one might possibly expect one or all of the following reactions.

1. (Oppositional factor hypothesis). There might be a reaction be-

tween the tissues of the style and the factors carried by the pollen. Such a reaction would account for most of the phenomena of cross- and self-sterility in *Nicotiana* (3) and a number of other genera. With simple extensions the same hypothesis can be applied successfully to polyploids (1).

2. There might be a reaction between the tissues of the style and the pollen tube, determined not by the factors carried by the pollen itself but by those of the plant on which it was borne. One might expect such a reaction to be much more likely between the pollen grain and the stigma, than between the pollen tube and the style.

3. The reaction of either (1) or (2) might influence the germination and growth of other pollen.

According to the oppositional factor hypothesis, the operation of self- and cross-sterility (in a diploid) would be explained as follows: Pollen grains carrying genetic factors identical with those of the style would be inhibited, and although they might germinate would not grow fast enough to cause fertilization. Plants of identical constitution for these essential factors would be completely cross-sterile and plants having one factor in common would be semi-compatible, although this partial sterility might be apparent only by genetical tests. For example, if a plant of the constitution S_1S_2 were crossed with a plant of the constitution S_1S_3, half of the pollen would carry S_1 and half S_3. The former would be inhibited by the action of the S_1 factor in the style and the resulting progeny would be of only two classes, S_1S_3 and S_2S_3.

The *Tradescantia* plants available for cytological examination consisted of a number of samples of natural populations. If the oppositional factor hypothesis as just outlined is responsible for the incompatibilities in these populations, there are a number of means by which it should be possible to test the matter cytologically, as follows:

1. There should be inter-fertile, intra-sterile classes. These were found as has been reported.

2. There should be two grades of cross-fertile matings, the fully compatible (type $S_1\ S_2 \times S_3\ S_4$) and the semi-compatible (type $S_1\ S_2 \times S_1\ S_3$) explained above. On this point the results are not conclusive. The smear technique employed does not make it possible to follow

the majority of pollen tubes after they have left the upper portion of the style. Compatibility may be measured in a rough manner by determining the percentage of pollen grains which germinate but in which the nucleus does not leave the grain promptly. Counts were made on styles smeared 8 hours after pollination for 30 different compatible matings in *T. gigantea*. The percentage of germinated grains whose nuclei had gone down the tube ranged from 24 to 76 per cent. The percentage formed a unimodal distribution, with over half of them falling between 40 and 60 per cent. This may possibly mean that all of the matings tested were semi-compatible with an expecta-

TABLE IV

LENGTHS OF POLLEN TUBES, IN μ, 30 MINUTES AFTER POLLINATION. FROM SMEARS OF SELF-STERILE AND CROSS-FERTILE POLLINATIONS IN TRADESCANTIA GIGANTEA

Length of pollen tubes (μ)													
0–24	25–	50–	75–	100–	125–	150–	175–	200–	225–	250–	275–	300–325	
2	1	14	9	2	2	....	....	....	....	....	....	....	plant no. 4W selfed
1	0	1	0	5	4	5	0	2	0	1	0	2	plant no. 5 × no. 4W

tion of 50 per cent, or it may mean that the oppositional factor hypothesis in its simplest form cannot be applied to *Tradescantia*.

3. In a semi-compatible mating there should be two main groups of pollen, the inhibited and the uninhibited, and they should occur in approximately equal numbers. Measurement of all the pollen tubes should show a bimodal distribution of tube length. The best data obtainable by the smear technique described are presented in table IV. It will be seen that there is no evidence for a bimodal ranking of the pollen tube lengths. This may possibly mean that the oppositional factor hypothesis will not account for the phenomena of cross-sterility in *Tradescantia*. On the other hand, the expected bimodality may possibly be obscured by modifying factors or by an interaction between the compatible and the incompatible pollen tubes.

In the absence of any genetical evidence, it does not seem profitable to discuss these and other possibilities at greater length. Briefly the cytological evidence is in line with the main requirements of the oppositional factor hypothesis, although it does not demonstrate the sharp difference between semi-compatible and fully compatible matings which would be expected on the simplest interpretation of that hypothesis.

Summary

1. Germinating pollen grains of *Tradescantia* are described as they appear in aceto-carmine smears of pollinated styles.

2. Many species of *Tradescantia* are self-sterile.

3. Compatible and incompatible pollination can be distinguished cytologically. The pollen grains germinate normally in the latter, but their growth is inhibited.

4. Cross-sterile pollinations cannot be distinguished quantitatively nor qualitatively from self-sterile pollinations.

5. Natural populations of diploid *Tradescantia* can be divided into intra-sterile, inter-fertile classes on the basis of pollen tube behavior.

6. The number of these classes in natural populations is found to be large.

7. The main facts revealed by this purely cytological examination are in line with the oppositional factor hypothesis although there is disagreement on a few minor points.

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ONTOGENY OF THE PRIMARY AXIS OF SOJA MAX

CONTRIBUTIONS FROM THE HULL BOTANICAL LABORATORY 452

WILLIS H. BELL

(WITH THIRTY-SEVEN FIGURES)

Introduction

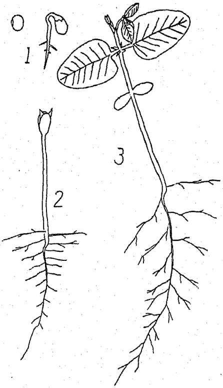
A voluminous literature dealing with nodule development and physiological relationships of the soy bean (*Soja max* Piper) has accumulated, but apparently the only strictly anatomical work has been a paper on the floral development by GUARD (4) and a brief seedling investigation by COMPTON (2). The present study has been undertaken to investigate the development of the primary axis. The variety used was the commercial Mammoth Yellow. PIPER and MORSE (6) have given the following description of this variety: "Plants stout, erect, bushy, maturing in about 145 days, pubescence gray; flowers white." At maturity the plants may be either bushy, with only a few internodes elongated, or vine-like, with more internodes elongated, depending upon physiological conditions of growth. The first leaves are simple and opposite and at right angles to the plane of the cotyledons. All other leaves are alternate and trifoliately compound. The flowers of this variety are in axillary racemes. The aerial portions of the plant are pubescent with simple hairs.

Investigation

The plants were grown under greenhouse conditions, and seedlings ranging from two to twenty days old were fixed for study. The stem and hypocotyl were fixed either in formal-acetic-alcohol or in chromo-acetic acid, and the root in chromo-acetic acid. The material was run up through alcohol and chloroform, imbedded in paraffin, sectioned at 12 μ, mounted serially, and stained with safranin and gentian violet. Drawings were made with the aid of a micro-projector and camera lucida.

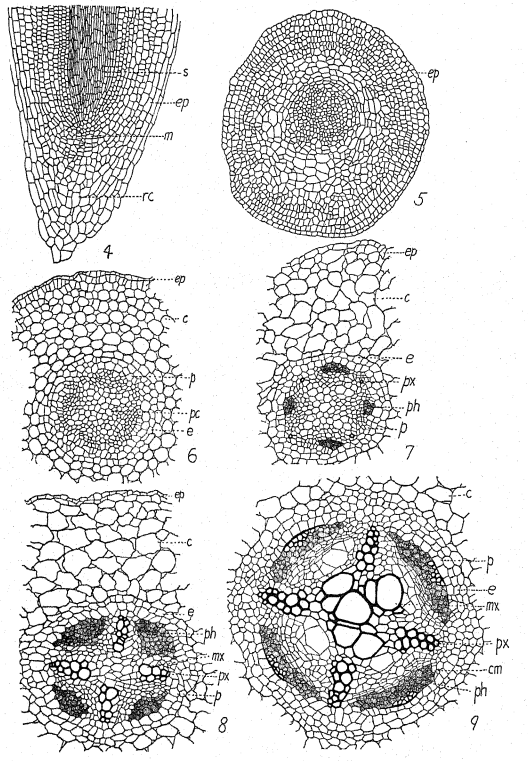
GERMINATION.—After imbibition of water the young primary root pushes through the seed coats and rapidly establishes itself as

an absorbing organ (fig. 1). The hypocotyl elongates, pulls the cotyledons up through the soil, and the seed coats are shed before the cotyledons appear at the surface of the ground (fig. 2). The cotyledons remain folded together as in *Phaseolus* until they are several centimeters above the surface of the soil. The epicotyl elongates but little until after the cotyledons unfold, and then it develops rapidly (fig. 3).



FIGS. 1–3.—Fig. 1, early stage in germination. Fig. 2, stage in germination after final elongation of hypocotyledonary arch. Fig. 3, young seedling with first true leaves fully developed.

PRIMARY ROOT.—The primary root at the completion of primary differentiation is a tetrarch radial protostele (fig. 9). It is differentiated from a meristematic group of cells at the tip of the hypocotyl (fig. 4). Development is according to JANCZEWSKI'S (5) "fourth angiospermous type," in which the stele, cortex, epidermis, and root cap do not arise from definite histogens but from a common group of meristematic cells (fig. 4). The root cap is the first tissue differentiated, and consists of several layers of loosely packed parenchyma cells overlying the root tip. The epidermis is uniseriate and develops



FIGS. 4–9.—Fig. 4, longitudinal section of root tip (*s*, stele; *ep*, epidermis; *m*, meristem; *rc*, root cap). Fig. 5, cross-section of primary root above root cap showing epidermis differentiated. Fig. 6, same at time of differentiation of stele and cortex (*c*, cortex; *p*, pericycle; *e*, endodermis; *pc*, procambium). Fig. 7, same showing first protoxylem and protophloem (*ph*). Fig. 8, same showing primary xylem partially differentiated (*px*, protoxylem; *mx*, metaxylem; *ph*, primary phloem). Fig. 9, same after cambial activity has been initiated (*cm*, cambium).

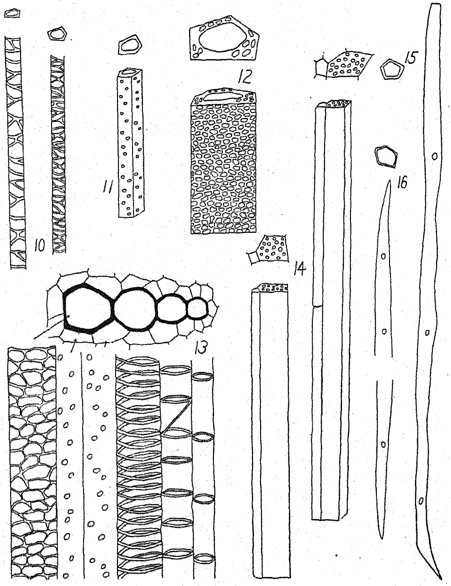
root hairs early in ontogeny (fig. 5). The cells of the cortex, except those of the endodermis, become large and loosely arranged with intercellular spaces while the cells of the stele are smaller and more compact (fig. 6). The primary xylem is exarch in its development, the first cells of the protoxylem differentiating from the procambium at four points adjacent to the pericycle (fig. 7). The one to three protoxylem vessels differentiated at each of the four points show spiral-reticulate markings on their walls (fig. 10). Metaxylem is matured progressively inward from the protoxylem points (fig. 8). The first metaxylem vessel segments have pitted walls with a single row of pits in each (fig. 11). The undifferentiated cells at the center of the primary root enlarge considerably and differentiate as metaxylem (fig. 9). The majority of these cells develop into short vessel segments with densely pitted walls (fig. 12). Some of the cells of this later developing metaxylem remain parenchymatous and ultimately differentiate into thick walled connective tissue.

The cells lying between the protoxylem points and adjacent to the pericycle differentiate centripetally as primary phloem (fig. 7). The first cells of the primary phloem remain parenchymatous and are crushed against the pericycle upon the initiation of cambial activity (fig. 9). The cells immediately beneath this parenchyma develop into phloem fibers. The remainder of the primary phloem consists of scattered parenchyma cells, sieve tubes, and companion cells. The sieve tube may be the same length as the companion cell or it may be equivalent in length to two companion cells (fig. 14). Cambial activity is initiated while the cells at the center of the root are still parenchymatous. At first activity is greatest immediately beneath the phloem, resulting in the crushing of the protophloem (fig. 9).

At about this time or somewhat earlier, the pericycle gives rise to secondary roots which dissolve their way through the cortex as they develop. The development of the primary body of the secondary root is identical with that of the primary root, except that ordinarily only one large metaxylem vessel instead of several is matured in the center of the root. The secondary root is usually smaller than the primary root, and may be diarch, triarch, or tetrarch.

HYPOCOTYL.—The hypocotyl as interpreted in this paper is that portion of the plant axis between the cotyledons and the true root.

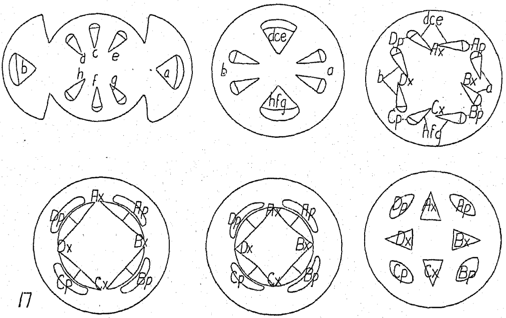
The lower portion of the hypocotyl is rootlike while the upper part is similar to the stem in the orientation of its tissues (figs. 19, 26). Upon germination the hypocotyl elongates as a result of meristematic

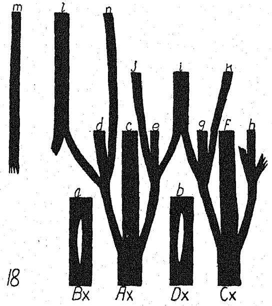


FIGS. 10–16.—Fig. 10, histology of protoxylem vessels of root. Fig. 11, histology of first metaxylem vessels differentiated in root. Fig. 12, histology of metaxylem vessel in center of root. Fig. 13, histology of primary xylem of stem. Fig. 14, sieve tubes and companion cells from root. Fig. 15, histology of phloem fibers of root. Fig. 16, immature pericyclic fiber from stem.

activity in the region of the hypocotyledonary arch (fig. 1). Procambial differentiation in the embryonic and elongating hypocotyl results in continuity of the procambium of the root and of the double

bundle to each cotyledon. Reorientation of the procambial strands in the lower part of the hypocotyledonary axis results in a transition

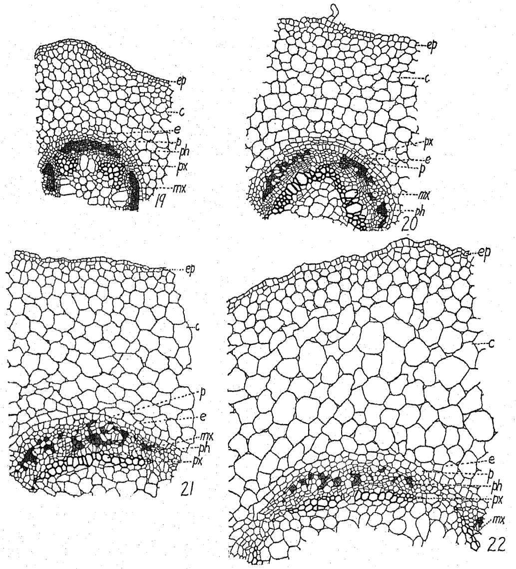




FIGS. 17, 18.—Fig. 17, diagrammatic cross-sections at different levels of the hypocotyl, illustrating method of root-stem transition and relationship of vascular supply of cotyledons and epicotyl to that of root. Fig. 18, diagram of main vascular supply of cotyledons and leaves at first three nodes.

between the radial protostelic structure below and the endarch collateral condition above. In this manner the four endarch collateral

bundles representing the traces to both cotyledons are differentiated against the tetrarch radial protostele of the root (figs. 17–26).



FIGS. 19–22.—Fig. 19, cross-section of hypocotyl just above level of root showing xylem groups about to bifurcate. Fig. 20, same showing siphonostele as a result of xylem divergence in root-stem transition. Fig. 21, same showing xylem divergences in contact by their metaxylem. Fig. 22, same showing xylem divergences entirely separated by parenchyma.

The exact manner of reorientation of the hypocotyledonary vascular system is shown in figures 17 and 18. The double bundle *a* of one cotyledon passes downward as two groups, which upon undergoing transition just above the root tie against the xylem *bx* and one wing of the phloem *bp* and *ap* of the root. The double bundle *b* to the

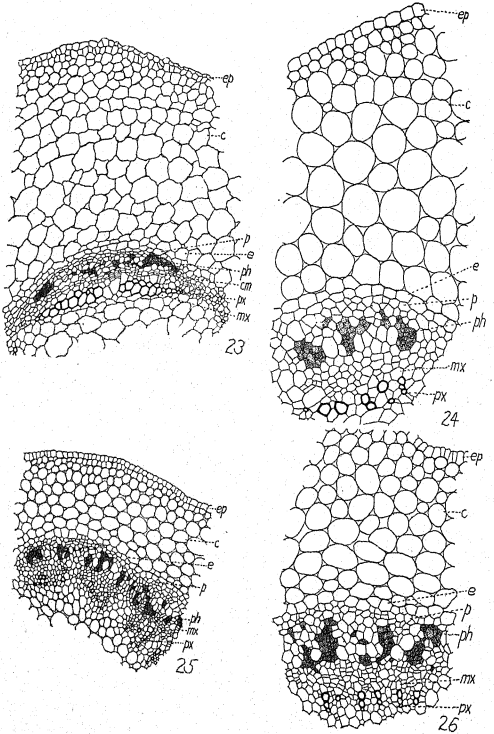
other cotyledon is differentiated against xylem *dx* and one wing of the phloem *cp* and *dp* of the root.

Cotyledons.—The cotyledons are sessile and at maturity are fleshy and oval in shape (figs. 3, 29). The veins of the cotyledons are divergences from the double bundle entering at the base. After maturation of the opposite leaves the cotyledons are dropped from the plant.

Epicotyl.—Prior to germination the epicotyl of the embryo has differentiated several foliage leaves. The first leaves, which are simple and opposite, are differentiated at right angles to the plane of the cotyledons. The leaf primordium arises as a conical crescentic divergence from the apical meristem, which because of meristematic activity broadens laterally at its base until the entire periphery of the growing point is involved. This lateral activity results in the differentiation of two stipules for each leaf. The leaf primordium elongates with the cells at its apex differentiating a blade and the cells at the base elongating to form the petiole (fig. 27).

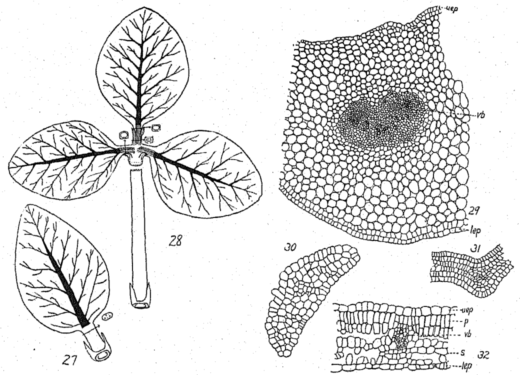
The primordia of the axillary flower buds are laid down at about the same time as are the associated leaf primordia. The procambial strands of the axillary bud are anastomosed with the downwardly differentiating strands of higher leaves at the node immediately below the point of divergence of the axillary bud.

In the development of the vascular system of the leaf the lateral bundles to the stipules converge with the median bundle at the base of the petiole. Meristematic activity continues in each stipule, resulting in the differentiation of a mesophyll. The veins to the stipule are divergences from the lateral bundle. Above the point of convergence of the median and lateral bundles the vascular system of the petiole appears stemlike, with several bundles in annular arrangement. Near the base of the lamina these bundles converge again to form a crescentic vascular ring. The blade is differentiated from a group of meristematic cells at the apical end of the leaf primordium (fig. 30). At first meristematic activity is general throughout, resulting in six to eight layers of cells (fig. 31). Differential growth eventually develops a blade with a single palisade layer and typical spongy parenchyma (fig. 32). Stomata are present in both the upper and lower epidermis but are more abundant in the lower.



FIGS. 23–26.—Fig. 23, cross-section of hypocotyl through transitional level showing cambium differentiation. Fig. 24, same showing primary xylem and phloem of transitional stage broken up into groups prior to endarch collateral orientation. Fig. 25, same showing both endarch and transitional xylem. Fig. 26, same after endarch collateral situation has been entirely attained.

The procambial strands which ultimately form the vascular skeleton of the blade are outward extensions of the median and lateral bundles of the petiole. In the leaf primordium the median bundle matures first, with the lateral bundles differentiating and maturing slightly later. The downward extensions of both are progressively matured as the hypocotyl and first internode elongate.



FIGS. 27–32.—Fig. 27, habit sketch of simple leaf. Fig. 28, same of compound leaf. Fig. 29, cross-section of a cotyledon (*uep*, upper epidermis; *vb*, median bundle; *lep*, lower epidermis). Fig. 30, cross-section of embryonic leaf. Fig. 31, cross-section of partially developed leaf prior to maturation of tissues. Fig. 32, cross-section of mature leaf (*p*, palisade parenchyma; *vb*, vascular bundle; *s*, spongy parenchyma).

One compound leaf is differentiated at right angles to the plane of the first pair of opposite simple leaves early in ontogeny (fig. 28). The development of this leaf is similar to that of the first true leaves just described. The median and lateral bundles of the trace are differentiated against the lateral bundles to the first leaves just above the cotyledonary plate. The stipules and petiole mature as just described but the petiole of the compound leaf undergoes much greater elongation. Instead of one meristematic group of cells at the tip of the primordium developing a blade, three regions of activity appear, giving rise to a trifoliate compound leaf, each leaflet receiving ap-

proximately one-third of the vascular supply of the petiole. Two small secondary stipules are developed as lateral divergences at the base of the terminal leaflet, and another pair immediately below the divergence of the two lateral leaflets (fig. 28). One small endarch bundle extends downward from each of these stipules and converges with the endarch groups to the leaflets which form the median and lateral bundles at the base of the petiole.

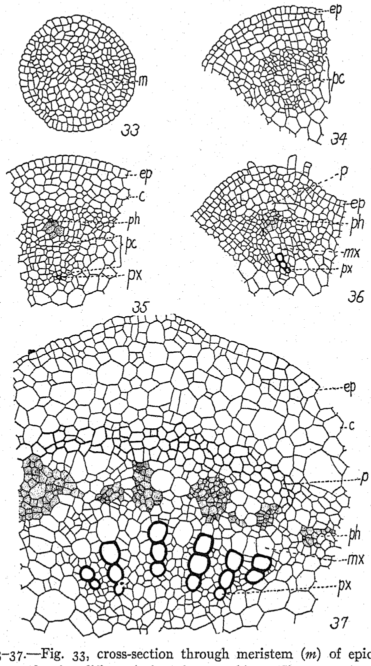
As seen in embryo, the median bundle *c* and the lateral bundles *d* and *e* of the trace to one of the opposite simple leaves pass downward and converge against the xylem *ax* and one wing of the phloem *ap* and *dp* of the root (figs. 17, 18). The trace *fgh* to the other opposite leaf converges with the xylem *cx* and one wing of the phloem *cp* and *bp* of the root. The trace *ijk* of the first alternate leaf is differentiated against the lateral bundles *e* and *g* of the opposite leaves at a level just above the cotyledonary plate (fig. 18). The trace *lmn* of the second alternate leaf converges against the lateral bundles *d* and *h* of the opposite leaves.

STEM.—The mature primary body consists of an endarch collateral dictyostele, pericycle, cortex, and epidermis (fig. 37). The pericyclic cells immediately external to the vascular bundles differentiate as fibers but in the region of the medullary rays remain parenchymatous. The primary vascular skeleton of the stem is a dictyostele consisting of the common bundles of the leaf traces.

The procambial strands may be recognized early by the greater density of the protoplasmic contents of their cells (fig. 34). The procambium adjacent to the undifferentiated parenchyma in the center of the stem matures as protoxylem (fig. 35). There are usually three strands of protoxylem (figs. 36, 37). The first strand shows widely separated annular markings (fig. 13) and is normally not collapsed at the time of maturity of the primary body. The later differentiated protoxylem strands show closer annular and spiral markings, the last formed consisting of tight spirals. Usually all the vessel segments have the histology of protoxylem but a few metaxylem vessels with pitted walls may develop. A large part of the primary xylem is parenchymatous at first and later matures as connective tissue.

Primary phloem is differentiated from the procambium in contact

with the pericycle and directly external to the primary xylem (figs. 35–37). The primary phloem consists of groups of sieve tubes and companion cells separated from each other by parenchyma (fig. 37).



FIGS. 33–37.—Fig. 33, cross-section through meristem (*m*) of epicotyl. Fig. 34, portion of stem showing differentiation of procambium. Fig. 35, cross-section of stem through region of vascular bundle at time of protoxylem differentiation. Fig. 36, same through vascular bundle. Fig. 37, same through median bundle to leaf.

The relationship between the sieve tubes and companion cells is similar to that in the root (fig. 14).

Summary

1. The root of the soy bean is developed according to JANCZEWSKI's "fourth angiospermous type," and at maturity is a tetrarch radial protostele which, as COMPTON (2) and DE CANDOLLE (1) point out, is common to many Leguminosae and especially to the Phaseoleae of which the soy bean is a member. A conical mass of parenchymatous cells comprises the root cap. The epidermis consists of a single layer of cells from which root hairs arise. The cortex is composed of several layers of parenchymatous cells and the endodermis and pericycle are each a single layer. Secondary roots arise from the pericycle. The anatomy of the secondary root is similar to that of the primary root except that usually only one large metaxylem vessel is matured in the center of the root instead of several. The protoxylem of the primary root consists of one to three spiral-reticulate vessels. The metaxylem matures in two stages and differentiates as pitted vessels and parenchyma. Part of the primary phloem is crushed at the initiation of cambial activity while the remainder differentiates as fibers and groups of sieve tubes and companion cells interspersed with parenchyma. A sieve tube may be the same length as the companion cell or may equal two companion cells in length.

2. Root-stem transition in the Phaseoleae is either low or intermediate, being low in the soy bean. As they pass upward the four exarch radial vascular groups of the root bifurcate and reorient as eight endarch collateral bundles.

3. The double traces of the cotyledons pass downward and converge against two vascular groups of the root in the same plane.

4. The cotyledons are fleshy photosynthetic leaves which drop off after maturation of the first pair of opposite leaves.

5. The first leaves are simple and opposite with a pair of stipules at the base of the petiole. The trace to each leaf is composed of three bundles which are differentiated downward against the vascular system of the root at the level of transition.

6. The later leaves are alternate and compound and their traces, consisting of three bundles, pass downward through two or more internodes before anastomosing with the lateral traces of other leaves.

7. The stem consists of an endarch collateral dictyostele, peri-

cycle, cortex, and epidermis. The primary vascular skeleton is a dictyostele consisting of the common bundles of the leaf traces. Usually all the vessel segments have the annular and spiral thickening of protoxylem but sometimes a few metaxylem vessels with pitted walls can be observed. A large part of the primary xylem remains parenchymatous and later matures as connective tissue. Primary phloem consists of sieve tubes and companion cells separated by parenchyma. They have the same histology as in the root. The pericycle immediately external to the vascular bundles differentiates as fibers but in the region of the medullary rays remains parenchymatous.

The writer takes pleasure in expressing his indebtedness to Dr. H. E. Hayward, who has given valuable suggestions and criticisms during the progress of this work.

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STEM STRUCTURE OF GRASSES ON THE JORNADA EXPERIMENTAL RANGE

R. H. CANFIELD

(WITH EIGHT FIGURES)

Introduction

The orthodox description of the grass family contains the phrase "stems usually hollow," which has been generally accepted as meaning that all mature grasses other than the genera of the Andropogoneae and Nazieae have hollow stems. Early in 1927, when certain grasses not members of either of these tribes were examined, they were found to have solid stems. Continued investigation showed that a very high percentage of the mature grasses native to the semiarid Jornada plain have solid internodes.

BEWS (1) states that the stems of grasses are usually hollow, but that a number of "types" have solid stems, including the Andropogoneae and many of the Paniceae. VAVILOV (6) also refers to a solid stemmed variety of *Agropyrum cristatum* Beauv., which he reports as being widely distributed over European and Asiatic Russia.

JORNADA EXPERIMENTAL RANGE.—This area is a branch of the Southwestern Forest and Range Experiment Station, and includes approximately 300 square miles of semi-arid lands located near Las Cruces, New Mexico, some 50 miles north of the Mexican border. The region is typical of the south-central New Mexico grasslands. The greater part of it is a flat to slightly rolling mesa with elevations ranging from 4100 to 4700 feet. In addition, there are about 73 square miles of mountainous country which include the foothills and western slopes of the San Andres Mountains. Elevations vary in this area from 4700 in the foothills to 8000 feet on the higher peaks.

CLIMATE.—Climatically the Jornada region is one of the most arid in the southwest. The average annual rainfall for the 17-year period covered by the Jornada Experimental Range precipitation records is 8.99 inches, with a maximum of 17.73 inches and a minimum of 3.54 inches. Annual rainfall for seven of the seventeen

years was above the average for the period, and for ten of the years was below the average.

Usually the first effective summer rains fall late in June or early in July. A general beginning of plant growth takes place after this rainfall and continues through a season of about 100 days. This period, which includes July, August, and September, is commonly referred to as the growing season. According to the Jornada records the average precipitation for the period of growth is 4.64 inches. Of the seventeen years included, six were above the average and eleven were below it. During this period of record the maximum summer seasonal was 8.53 inches and the minimum was 2.34 inches.

The average date of the last spring frost is April 9, and the average date of the first fall frost is October 26; 200 days is the average frostless period. Temperatures as high as 106° F. and as low as −8° F. sometimes occur. The average wind velocity is 7.1 miles per hour.[1]

Recent measurements made at the Jornada headquarters show that a 0.5-inch daily evaporation loss from a free water surface is common during the summer and early fall months. A combination of hot dry winds and low relative humidity, sometimes as low as 10 per cent, gives a high rate of evaporation and a correspondingly high rate of transpiration in the vegetation.

The most prominent climatic characteristic is of course the ever-recurring periods of insufficient soil moisture. SHANTZ (5) states: "True drought can occur only when the available soil water has been exhausted." It is also accepted as fact that the individual plant represents the sum of all the factors which have affected its growth, of which available water is one. Drought is therefore a specific condition which can be detected in observable effects on the vegetation. The character of the effects from true drought are such as would, if continued for a sufficient time, eliminate the plants concerned from the area under consideration.

The writer (3) has previously offered a definition of drought which is believed to be sufficiently precise for range purposes. Drought was defined as a condition in which the water requirements of the established dominant plants of the highest successional stage exceed

[1] The climatic data are taken from the records of the New Mexico A. & M. College, located 20 miles south of the Jornada Experimental Range.

the available water supply, and which, if continued beyond the life span of these plants and beyond the duration of the viability of their seeds, would result in the extinction of the species. Drought in the Jornada region may be separated into two classes, general and stratified. General drought affects the whole of the soil profile utilized by plants; stratified drought occurs in horizontal layers within the soil profile and affects only the plants which have their roots mainly in the dry layer.

In connection with the climatic conditions, the sand storms which occur with unfailing regularity during the spring and early summer months are of importance. In addition to the damage incident to erosion and deposition of soil, the abrasive action of the sand blast increases the difficulties under which the succulent vegetation makes its struggle for an existence.

VEGETATION.—In spite of the climatic conditions, the Jornada grasslands produce abundant crops of forage. Figures 1 and 2 show typical range conditions in a year of average seasonal rainfall. The native vegetation consists of herbaceous plants and shrubs which are capable of maintaining an existence under conditions of low rainfall, high temperature, and a dry atmosphere. The more common plants take advantage of periods of abundant moisture, either by completing their growth in one usually short, wet period or by alternating rapid growth with dormancy through the succeeding wet and dry periods until maturity is attained.

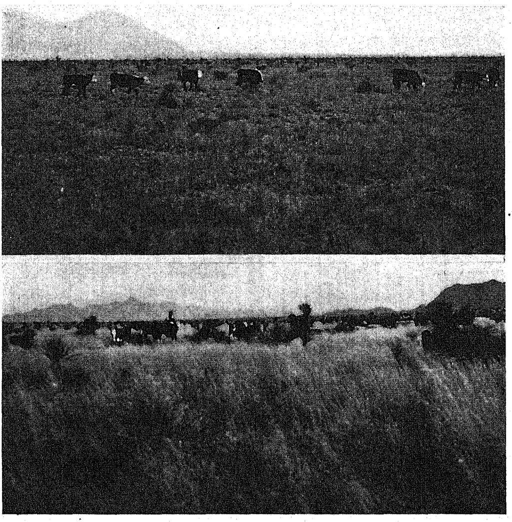
CANFIELD (4) separates the grasses occurring in this region into four divisions or classes, based on the order of their development. The influence of the stem structure is apparent in these groups.

One class is composed of the annuals which complete growth early in the season, during a period of available moisture when the temperatures are low and the relative humidity is high enough to retard transpiration. The proportions of grasses with solid and with hollow stems are about equal in frequency in this group; nevertheless the solid stemmed species contribute materially to the plant cover while the hollow stemmed species occur infrequently.

A second group, composed chiefly of the perennial *Aristida* and *Hilaria mutica*, produce a mature crop of plants at any time during the frostless season when an unbroken period of available moisture

of sufficient length occurs. A spring crop of these grasses followed by one or more summer crops is not an uncommon event. The members of this group are solid stemmed without exception.

A third group, represented by *Bouteloua eriopoda* and species of *Sporobolus* and *Muhlenbergia*, occurring on the well drained sandy or



FIGS. 1, 2.—Fig. 1 (above), range in early summer before growing season begins. Fig. 2 (below), range in fall after a good growing season.

gravelly soils, make their growth by stages. These grasses develop rapidly during periods of abundant moisture and high night temperature, although usually they are unable to attain maturity in one unbroken period. In the droughts which usually intervene between the rains, these grasses suspend activity and remain in a dormant condition, bursting into growth when the next soil-saturating rain

comes. An almost immediate response to moisture is an outstanding characteristic of this group. Two members of the third group, *Bouteloua eriopoda* and *Muhlenbergia porteri*, are notable because of their true perennial habit of growth. Stems of these grasses remain green throughout the winter and leaf out again the following year. All species falling into this group are solid stemmed.

A fourth group contains *Koeleria cristata* and other grasses, most of them hollow stemmed, which are nearing the lower limits of their ranges. These species occur only in the more favorable situations, such as depressions, intermittent stream beds, and sheltered spots where moisture is present for longer than the usual periods.

Artificial planting of foreign grasses has resulted in failure. Grasses from distant arid lands which come highly recommended have not survived a single season.

Investigation

FIELD METHODS.—Field investigations on the stem structure of grasses were started in the late summer of 1927, and carried through each succeeding summer and fall up to and including the 1930 seasons. Mature plants of the various species were selected and a well developed culm was taken from the specimen. The culms were cut transversely through at a point about equidistant from two nodes, and an examination made with a hand lens. Should a stem be of very small diameter, or if for any other reason there was doubt as to the determination of the nature of the structure with the hand lens, material was selected in the field and preserved for microscopic study in the laboratory.

Identification of the species was established by making a study of each grass and comparing specimens collected in the field with authentic specimens in the Jornada Experimental Range herbarium. All specimens in this herbarium have been identified by the United States Bureau of Plant Industry.

LABORATORY METHODS.—Material collected in the field for laboratory use was immediately placed in a small individual vial of formalin acetic alcohol. The vial was temporarily labeled and numbered in the field. Later each specimen received a permanent label, placed inside the vial, which was then corked and sealed with paraffin.

Considerable difficulty was encountered in preparing the material for imbedding and sectioning. The high silicon content of the grass stems was a serious obstacle which prevented their successful sectioning. In attempts to remove the silicon, the material was immersed in solutions of hydrofluoric acid of different concentrations for varying periods of time. At last it was found that the use of 100 per cent hydrofluoric acid with an immersion period of one week was about right for the greater part of the material.

Further difficulties developed in sectioning. Good sections could not be secured by the usual alcohol stages of dehydrating and the usual paraffin method of imbedding. When the material was run through the alcohols using a 5 per cent solution as a beginning and advancing by 5 per cent stages to absolute, however, the result was satisfactory. The stems were then imbedded in celloidin.

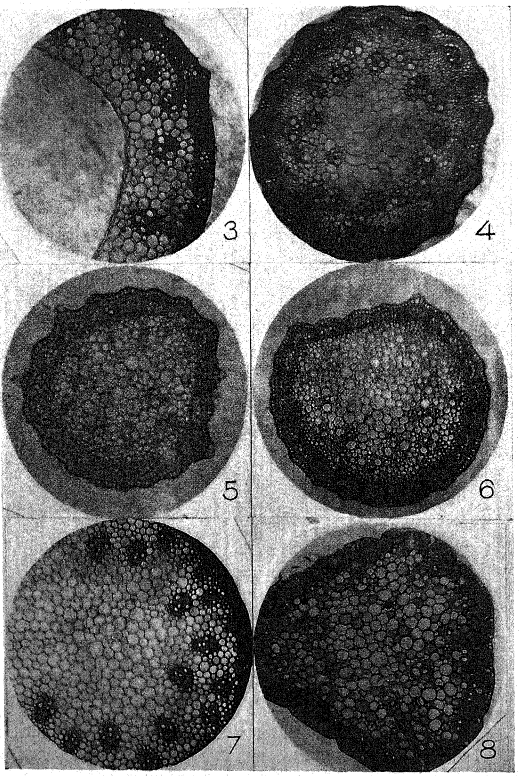
The formula used in making transverse sections of grass stems is as follows:

1. Kill in formalin acetic alcohol solution.
2. Immerse in 100% HF one week.
3. Wash in running water 24 hours.
4. Dehydrate using alcohol stages 5% to 100%, 5% in each step, changing at 4-hour intervals.
5. Imbed in celloidin, 2% to 16%, 12 hours in each solution.
6. Stain sections in iron-alum haematoxylin.
7. Mount in thin balsam.

Figures 3–7 show the stem structure of a few representative specimens of solid and hollow stemmed grasses. The cross-sections were made at a point about equidistant from two nodes and are typical of the species which they represent. In making the slides from which the photomicrographs were taken, it was necessary to cut the sections much thicker than usual to avoid tearing the thin walled cells in the central portion of the stems. These cells had become fragile from exposure to the strong acid used in removing the silicon.

Since it was desired that the photographs show the cell structure in the central parts of the stems, it was necessary in most instances to throw the heavy walled cells of the outer portions of the stems out of focus.

No attempt is made in this paper to enter into a detailed discus-



FIGS. 3–8.—Transverse sections of grasses: Fig. 3, *Leptochloa fascicularis*, hollow stemmed annual grass occurring only in favored locations where moisture is available for longer than the usual periods. Note large central cavity. Fig. 4, *Hilaria mutica*, solid stemmed perennial grass (solid stems would be expected in this genus) occurring only on heavy clay soils and thriving only on areas subjected to periodic summer flooding. Fig. 5, *Scleropogon brevifolius*, perennial grass described by CAMPBELL (2) as a pioneer occurring on denuded clay flats. Fig. 6, *Muhlenbergia porteri*, perennial grass once common but now sparse, probably because stems are perennially green and therefore much sought by grazing animals. Fig. 7, *Sporobolus cryptandrus*, solid stemmed perennial grass occurring on dry sandy ridges and plains. Fig. 8, *Cyperus uniflorus*, a sedge requiring moist or wet soil as its habitat, included here to show similarity in stem structure to grasses of the semi-arid mesas.

sion of the differences in cell structure and cell arrangement which are apparent under the microscope. However, the thin walled cells of the central portions of the solid stemmed grasses and the arrangement of the vascular bundles are interesting items.

In the following list of species, all the grasses known to occur on the Jornada Experimental Range have been classified as to whether the stems are solid or hollow.

Solid stemmed annuals (7 species)

Nazieae, Tribe III
- Nazia aliena (Spreng.) Buckl.
- N. racemosa (L.) Kuntze

Paniceae, Tribe V
- Cenchrus pauciflorus Benth.

Agrostideae, Tribe VIII
- Aristida adscensionis L.

Chlorideae, Tribe X
- Bouteloua aristidoides (H.B.K.) Griseb.
- B. barbata Lag.
- B. parryi (Fourn.) Griff.

Hollow stemmed annuals (8 species)

Paniceae, Tribe V
- Panicum barbipulvinatum Nash
- P. hallii Vasey
- P. hirticaule Presl.

Chlorideae, Tribe X
- Leptochloa fascicularis A. Gray (fig. 3)

Festuceae, Tribe XI
- Eragrostis pilosa (L.) Beauv.
- Festuca octoflora Walt.
- F. octoflora hirtella Piper
- Munroa squarrosa (Nutt.) Torr.

Solid stemmed perennials (44 species)

Andropogoneae, Tribe II
- Andropogon saccharoides Swartz

Nazieae, Tribe III
- Hilaria mutica (Buckl.) Benth. (fig. 4)

Paniceae, Tribe V
- Echinochloa crus-galli (L.) Beauv.
- E. crus-galli mitis (Pursh) Peterm.
- Panicum obtusum H.B.K.
- Paspalum distichum L.
- Chaetochloa macrostachya (H.B.K.) Scribn. & Merr.

Agrostideae, Tribe VIII
- Aristida divaricata Humb. & Bonpl.
- A. glauca (Nees) Walp.
- A. havardii Vasey
- A. longiseta robusta Merr.
- A. pansa Woot. & Standl.
- A. purpurea Nutt.
- A. schiediana Trin. & Rupr.
- Epicampes emersleyi (Vasey) Hitchc.
- E. rigens Benth.
- Lycurus phleoides H.B.K.
- Muhlenbergia arenicola Buckl.
- M. monticola Buckl.
- M. pauciflora Buckl.
- M. porteri Scribn. (fig. 6)
- M. repens (Presl.) Hitchc.
- Oryzopsis hymenioides (Roem. & Schult.) Ricker
- Sporobolus airoides Torr.
- S. asperifolius (Nees & Meyen) Thurb.
- S. auriculatus. Vasey
- S. cryptandrus (Torr.) A. Gray (fig. 7)
- S. flexuosus (Thurb.) Rydb.
- S. giganteus Nash
- S. nealleyi Vasey
- Stipa eminens Cav.

Chlorideae, Tribe X
- Bouteloua breviseta Vasey
- B. curtipendula (Mich.) Torr.
- B. eriopoda Torr.

B. gracilis (H.B.K.) Lag.
B. hirsuta Lag.
Chloris virgata Sw.
Leptochloa dubia (H.B.K.) Nees

Festuceae, Tribe XI
Distichlis spicata (L.) Greene
Eragrostis cilianensis (All.) Link
E. erosa Scribn.
Scleropogon brevifolius Phil. (fig. 5)
Triodia mutica (Torr.) Scribn.
T. pilosa (Buckl.) Merr.

HOLLOW STEMMED PERENNIALS (10 SPECIES)

Paniceae, Tribe V
Leptoloma cognatum (Schult.) Chase
Valota saccharata (Buckl.) Chase

Agrostideae, Tribe VIII
Agrostis verticillata Vill.
Muhlenbergia rigida (H.B.K.) Kunth
Stipa columbiana Macoun
S. scribneri Vasey

Aveneae, Tribe IX
Koeleria cristata (L.) Pers.

Chlorideae, Tribe X
Capriola dactylon (L.) Kuntze

Festuceae, Tribe XI
Pappophorum wrightii S. Wats.
Triodia pulchella H.B.K.

Discussion

The terms solid stemmed and hollow stemmed refer to the condition of the grass stems at maturity. Due consideration has been given to the natural condition of the growing stem, and only mature stems have been used in this study. It is a recognized fact that nearly all grass culms have no hollow space in the internodes during the early periods of growth. This condition is especially true of the meristematic regions just above the nodes.

This study of the Jornada grasses includes seven tribes which are represented by 30 genera and 69 species. Of the total number, 51 species (74 per cent) have solid stems while only 18 species (26 per cent) have hollow stems.

ANNUAL GRASSES.—The annual grasses consist of 15 species of which seven are solid stemmed and eight are hollow stemmed. Annual grasses, because of their scattered occurrence and their short stature, constitute only a small fraction of the Jornada forage crop.

The solid stemmed annual species make their growth at any time during the growing season when the top layer of soil furnishes moisture for a period of sufficient length for them to complete development. These grasses belong in the class which completes growth in one unbroken interval. Although the rate of development may be perceptibly slowed down at times, either by a decrease in the depth of the moist top layer of soil or by an increase in the rate of transpiration to a point which is almost equal to the rate of absorption, in no observed instance can these grasses be said to have passed through a period of dormancy and to have resumed their growth with the return of favorable conditions.

The hollow stemmed annual grasses rely on a rapid development from germination to maturity for the maintenance of the species. Their period of growth generally is restricted to a short rainy interval in the early part of the season, at which time the days are cool and the relative humidity is high. However, an occasional plant may be found later in the season in sheltered spots or in catch basins, where the required climatic conditions are maintained by unusual factors beyond the regular season.

There is overwhelming evidence in practically all the species which have been observed that the hollow stemmed annual grasses of this semi-arid region thrive only under climatic conditions which are somewhat similar to those of the humid regions where the grasses are chiefly hollow stemmed.

PERENNIAL GRASSES.—The perennials are represented by 55 species, of which 45 (82 per cent) are solid stemmed while only 10 species (18 per cent) are hollow stemmed.

Native perennial grasses produce the major part of all the forage

on Southwestern plains. In the Jornada region the species of five genera, *Bouteloua*, *Sporobolus*, *Aristida*, *Hilaria*, and *Scleropogon*, furnish 85 per cent or more of the forage derived from grasses. All the species representing these genera are able to withstand to a marked degree protracted drought with grazing. They have the ability to exist in the less favorable situations, as well as in the more favorable ones. Observed representatives of these genera native to this region are without exception solid stemmed grasses.

The hollow stemmed perennial grasses furnish very little forage. They are sparsely scattered throughout the region, being restricted to the more favorable sites. Even in these more favorable locations, such as the bottoms of intermittent streams or the higher elevations where precipitation is greater, the hollow stemmed species generally exist under the protection of some jutting rock or in the shade of some hardy shrub.

Summary

1. In the species of grasses observed, the types of stem structure in the Jornada Range grasses apparently reflect the moisture requirements and the periods of growth.

2. The solid stem is characteristic of the grasses which are apparently best able to survive under the semi-arid conditions of the Jornada region.

3. Almost three-fourths (74 per cent) of the grasses collected have solid stems.

4. Tribes commonly reported as having solid stems, Andropogoneae and Nazieae, are represented by one genus each.

5. Solid stemmed perennial grasses produce 85 per cent or more of the forage.

6. Hollow stemmed grasses either grow only in the more favorable locations or escape drought by completing their growth during a few weeks of highly favorable moisture conditions.

7. Hollow stemmed grasses have not the ability to withstand the long dry periods.

8. There are strong indications that the solid stem is an index which may be employed in the selection of grasses for introduction into semi-arid regions.

The writer acknowledges his indebtedness to Mrs. AGNES CHASE, of the Bureau of Plant Industry; to W. A. DAYTON, of the U.S. Forest Service; to Dr. H. H. CASTLE, of Yale University; and to the Southwestern Forest and Range Experiment Station.

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OVULE AND EMBRYO SAC OF PLUMBAGO CAPENSIS

ARTHUR W. HAUPT

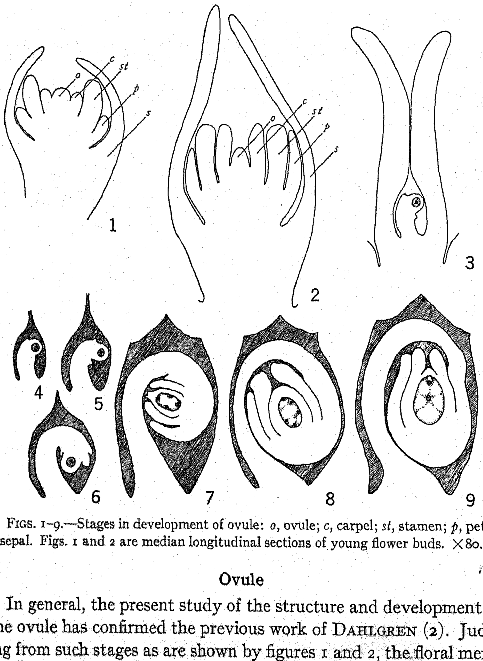
(WITH TWENTY-EIGHT FIGURES)

Introduction

Plumbago capensis is a South African shrub cultivated in greenhouses and extensively grown in the open in southern California. It produces a great profusion of azure blue or white flowers throughout the summer and autumn. Flowers in all stages of development may be collected from the same plant at any time during the blooming period, as new spikes continue to be formed. Although interest in the present investigation was confined to the ovule and embryo sac, it was found unnecessary to remove the ovule from the ovary, or even to remove the ovary from the flower, in order to prepare it for sectioning in paraffin. Whole flower buds, in the case of the smallest ones, were dropped into the fixing fluid, while in handling larger buds and opening flowers, the upper portions of the calyx and corolla were cut away so as to facilitate the entrance of the fixing fluid. The fixing agents giving the best results were formalin-acetic-alcohol and Nawaschin's fluid.

The only previous investigation of importance on the Plumbaginaceae is that of DAHLGREN (1), who in 1915 reported in a preliminary announcement the discovery of a new type of embryo sac in *Plumbagella*. This was followed the next year (2) by a full account of studies made on sixteen species of Plumbaginaceae, representing the following genera: *Acantholimon*, *Armeria*, *Goniolimon*, *Limoniastrum*, *Statice*, *Ceratostigma*, *Plumbago*, and *Plumbagella*. Of these, the first five genera, belonging to the Staticeae, were found by DAHLGREN to exhibit a normal 8-nucleate embryo sac of the *Lilium* type, while the last three genera, belonging to the Plumbagineae, were all found to possess a 4-nucleate sac of a unique and hitherto undescribed type. He investigated *Ceratostigma plumbaginoides*, *Plumbagella micrantha*, and three species of *Plumbago*, *P. capensis*, *P. pulchella*, and *P. zeylanica*. *Plumbagella* was studied in greatest detail.

I have examined only *Plumbago capensis*, but have made a thorough study and can state positively that this species does not exhibit the kind of embryo sac said by DAHLGREN to be characteristic of the three genera of Plumbagineae upon which he worked, but possesses a remarkable type which has not been described in any other angiosperm.



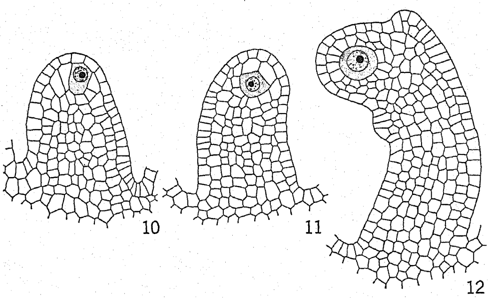
FIGS. 1–9.—Stages in development of ovule: *o*, ovule; *c*, carpel; *st*, stamen; *p*, petal; *s*, sepal. Figs. 1 and 2 are median longitudinal sections of young flower buds. ×80.

Ovule

In general, the present study of the structure and development of the ovule has confirmed the previous work of DAHLGREN (2). Judging from such stages as are shown by figures 1 and 2, the floral members seem to arise in acropetal succession. Earlier stages were not available. Following the appearance of the carpels, the apical

meristem of the floral axis becomes transformed directly into the single ovule which is characteristic of the Plumbaginaceae. Thus the ovule is strictly cauline in origin. The carpels develop around the ovule as a zone of tissue with five free tips. At first the style is hollow but later it becomes solid.

The ovule primordium has the form of a conical elevation consisting of uniform undifferentiated cells (fig. 2). At first it grows slowly and evenly, but soon a more rapid growth on one side causes the



FIGS. 10–12.—Longitudinal sections of young ovules showing formation of megaspore mother cell: fig. 10, archesporial cell (shaded); fig. 11, archesporial cell given rise to megaspore mother cell (shaded) and two parietal cells; fig. 12, megaspore mother cell (shaded) overlain by layer of parietal cells. ×400.

young ovule to bend in the opposite direction. The inner integument now arises as an annular outgrowth at the base of the nucellus; and as the curvature of the ovule becomes more pronounced through the elongation of the funiculus, the outer integument appears (figs. 3–6). The ovule is now inverted, the nucellus being directed downward. As development proceeds, however, the curvature of the ovule continues until it has turned completely over, the micropyle pointing upward (figs. 7–9).

The formation of the megaspore mother cell is similar to that described by DAHLGREN (2) for *Plumbago zeylanica*. The archesporium, which is hypodermal in origin, appears while the ovule is still very young, always before the integuments arise (fig. 10). Frequent-

ly the archesporium involves two cells (fig. 27) but as a rule only one is present. Since an archesporium consisting of more than one cell is confined chiefly to certain primitive families of Archichlamydeae and to other isolated cases, its occasional occurrence in *Plumbago* represents a primitive character.

The archesporial cell divides by a periclinal wall to form a primary parietal cell and the megaspore mother cell, an anticlinal division soon taking place in the former (fig. 11). By the formation of additional anticlinal walls, a layer of parietal cells arises beneath the epidermis of the nucellus (fig. 12), and by further periclinal divisions this is soon increased to two layers. In view of the fact that the suppression of parietal tissue in the ovule is nearly universal among the Sympetalae, its occurrence in *Plumbago* is especially noteworthy.

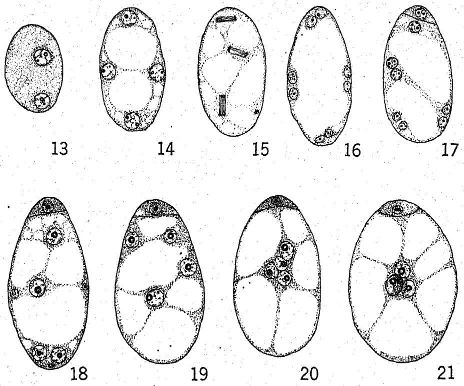
Embryo sac

As DAHLGREN (2) found in all of the species of Plumbaginaceae which he investigated, the megaspore mother cell, like that of *Lilium*, develops directly into the embryo sac, no linear tetrad of megaspores being formed. After the mother cell enlarges considerably, its nucleus undergoes the heterotypic mitosis (fig. 13). The homoeotypic mitosis, immediately following, results in the formation of four free nuclei arranged so that one is at each end of the embryo sac and one is at each side (fig. 14). This crosslike arrangement of the megaspore nuclei is very characteristic. By this time the embryo sac has become conspicuously vacuolate. As DAHLGREN found in the three species of *Plumbago* which he studied, no vacuole is formed in the binucleate stage.

Simultaneously the four megaspore nuclei now undergo a division, resulting in the formation of an 8-nucleate embryo sac (fig. 15). The daughter nuclei remain together in pairs, so that their orientation is the same as that shown by the four megaspore nuclei of the preceding stage (fig. 16). Instances of embryo sacs with eight nuclei were not so frequently observed as those with four nuclei, but the finding of several cases like the one shown in figure 16, and of one case where four mitotic figures were present (fig. 15), definitely establishes the fact that an 8-nucleate stage is reached.

The egg of *Plumbago capensis* is organized from one member of the

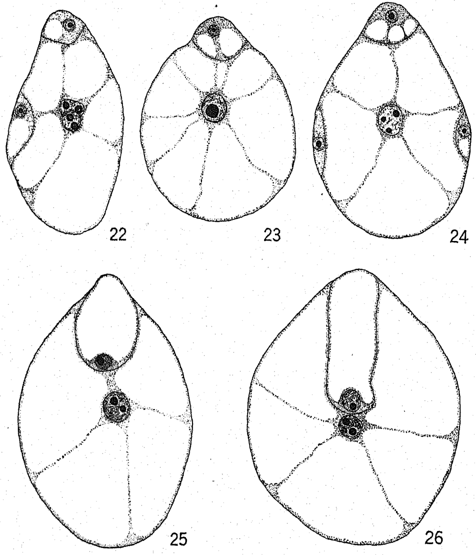
micropylar pair of nuclei present in the 8-nucleate stage, and is thus removed from the megaspore nucleus by only one division. The egg nucleus, together with a small amount of cytoplasm, is cut off from the rest of the embryo sac by a thin membrane (fig. 17). It remains as a small lenticular cell at the micropylar end of the sac until changes have taken place elsewhere. No synergids are differentiated. After the egg is organized, the other seven nuclei are left free in the



FIGS. 13–21.—Early stages in development of embryo sac: fig. 13, 2-nucleate stage; fig. 14, 4-nucleate stage; fig. 15, 4 nuclei dividing (one of the mitotic figures is oriented at right angles to plane in which section was cut); fig. 16, 8-nucleate stage; fig. 17, cutting off of egg; fig. 18, differentiation of the four polar nuclei; figs. 19, 20, fusion of polar nuclei. ×400.

general cytoplasm of the embryo sac. Four of these free nuclei increase slightly in size and gradually approach one another, functioning as polar nuclei, while as a rule the three remaining nuclei degenerate (fig. 18). Embryo sacs containing four large free nuclei and a small deeply staining cell at the micropylar end were very commonly encountered in the preparations (fig. 19), but their interpretation was not possible until stages intermediate between this one and the equally common 4-nucleate stage were found.

The four polar nuclei eventually come in contact near the center of the embryo sac and soon fuse (figs. 20, 21). Many of the preparations showed four nuclei about to unite, and where fewer were seen in close contact it was apparent that some had already fused (fig. 22).



FIGS. 22–26.—Later stages in development of embryo sac, with egg at micropylar end and fusion nucleus in center. In figs. 22 and 24 persistent lateral cells are formed from nuclei which ordinarily disappear in an earlier stage. Fig. 26 shows a mature embryo sac ready for fertilization. ×400.

A single large primary endosperm nucleus in an embryo sac containing an egg still immature is a stage which was frequently encountered (figs. 23, 24). Of the four fusing nuclei, one is the second member of the pair originally present at the micropylar end of the embryo sac,

but it could not be determined with certainty that each of the other three polar nuclei represents a single member of each of the other three pairs present in the 8-nucleate stage. Presumably, however, this is the case.

As previously stated, three of the eight nuclei formed in the embryo sac usually degenerate. Except for their position, they might be said to correspond to the antipodal nuclei of a normal 8-nucleate sac. While the degeneration of these nuclei is the rule, occasionally one, two, or even all three of them may persist and become organized as small naked cells. In figure 22 one such cell is present, while in figure 24 there are two. These persistent nuclei, which always seem to occupy a parietal position, are cut off by a membrane with a small amount of cytoplasm, as occurs when the egg is formed. In fact, often one or more of these cells may later enlarge and become egglike in appearance, suggesting that the embryo sac of *Plumbago* may have been derived phylogenetically from one in which each of the four megaspore nuclei gave rise to one egg and one polar nucleus. This interpretation is suggested by the situation in the Penaeaceae (6) and in *Euphorbia procera* and *E. palustris* (3, 4, 5), where by an additional division four peripheral groups of four nuclei each are formed, each group giving rise to a polar nucleus and to three cells resembling an egg apparatus. As in *Plumbago*, the primary endosperm nucleus results from the fusion of the four polar nuclei.

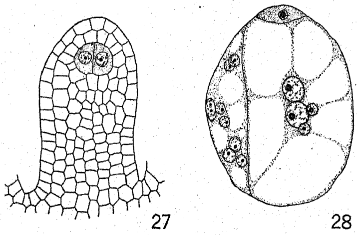
With the fusion of the four polar nuclei consummated, the egg undergoes a marked enlargement. Up to this point the nucleus lies at the upper end of the egg, the cytoplasm being vacuolate below; but with the enlargement of the egg the nucleus moves to its lower end and a large central vacuole fills its greater portion (figs. 25, 26). The fusion nucleus is situated immediately below the egg and rather near the center of the embryo sac. Except in rare cases, the mature embryo sac of *Plumbago capensis* contains only the egg and the fusion nucleus.

Fertilization was not studied, since in none of the flowers sectioned had pollination taken place. This was really an advantage, since it eliminated the possibility of any of the nuclei found in the embryo sac being male. Pollination is an infrequent occurrence, and as a result seeds are seldom formed. The flowers are sometimes visited by hawk

moths, which effect pollination. This was demonstrated by observing that flowers so visited later produced seed while other flowers did not. Artificial pollination is always successful. A number of seedlings were raised from seeds produced in this way. It was thought not necessary to artificially pollinate a number of flowers so that the details of fertilization could be studied, since DAHLGREN (2) has already given a full account of double fertilization, embryogeny, and endosperm formation.

Abnormalities

The presence of two megaspore mother cells is of such frequent occurrence that it can hardly be called an abnormality (fig. 27).



FIGS. 27, 28.—Unusual stages: fig. 27, two megaspore mother cells; fig. 28, two embryo sacs in same ovule. ×400.

Ordinarily only one gives rise to an embryo sac, but rarely both mother cells may function (fig. 28). In such cases one of the embryo sacs is much smaller than the other and somewhat tardy in its development. Several instances were seen of two embryo sacs in the same ovule, but separated from each other by several layers of nucellar tissue; and in one or two cases two ovules, partially grown together, were found in the same ovary. DAHLGREN (2) found a single case of two ovules in the same ovary in all three species of *Plumbago* which he investigated.

Discussion

DAHLGREN'S work was done chiefly on *Plumbagella*, but he found a similar type of embryo sac development in *Plumbago* and in *Cerato-*

stigma. His series of stages up to the 4-nucleate stage is in close agreement with mine, except that vacuolization in *Plumbagella* begins at the binucleate stage. Similarly DAHLGREN found in all three genera that the mature embryo sac possesses only an egg and a fusion nucleus. It is only in the development of the mature sac from the 4-nucleate stage that his account and mine differ.

In all three of the genera of Plumbagineae investigated, DAHLGREN (2) found that normally no further nuclear divisions occur beyond the 4-nucleate stage. The nucleus lying nearest the micropyle directly becomes the egg, the one next to the chalaza forms a single antipodal cell which soon degenerates, while the other two, representing the polar nuclei, later fuse to form the primary endosperm nucleus. Synergids are entirely absent. DAHLGREN'S figures 52–58, illustrating this development in *Plumbagella,* are much more convincing than his figures 61–72 of *Plumbago.*

Moreover DAHLGREN found certain "aberrant" cases, in both *Plumbago* and *Ceratostigma,* which he interprets on the basis of the development described for *Plumbagella,* but which would seem to be merely stages in such a developmental series as presented here for *Plumbago capensis.* Thus his figures 68 and 71 show embryo sacs of *P. zeylanica* with an egg and three fusing nuclei. In figure 69 a chalazal megaspore nucleus has divided to form two small nuclei, a large fusion nucleus being present near the egg. In figure 72 the fusion of a small nucleus (which DAHLGREN calls the "antipodal nucleus") with the primary endosperm nucleus is shown. In no case, however, did DAHLGREN find four nuclei fusing, but twice in *Ceratostigma* and once in *Plumbago zeylanica* young embryo sacs were encountered which contained, besides the newly formed egg, four free nuclei. In fact, a single 8-nucleate embryo sac (represented by figure 81) was seen in *Ceratostigma,* the occurrence of which DAHLGREN regards as atavistic. The cases just mentioned, considered by DAHLGREN to be anomalous, seem to indicate on the contrary that the embryo sac of *Plumbago* and *Ceratostigma* contain more than four nuclei and that more than two nuclei enter into the formation of the fusion nucleus.

Especially in *Ceratostigma,* but also in *Plumbago,* DAHLGREN (2) found anomalous embryo sacs with egglike cells present in addition to the true egg and the fusion nucleus. In *Plumbagella* such cases

were rare. An egglike cell at the chalazal end of the sac of *Plumbagella* is shown by figure 59, and in *Plumbago zeylanica* by figure 73. Figures 78 and 79 represent embryo sacs of *Ceratostigma*, each with a laterally situated egglike cell which DAHLGREN interprets as an antipodal which has assumed an unusual position and changed appearance. His figure 82 shows a similar case in *P. capensis*, while in figure 75 an egglike cell lies next to the egg as if it were a synergid. All of these cases may be explained as cell formation from persistent nuclei which normally degenerate; they are similar to those represented by figures 22 and 24 of the present paper.

DAHLGREN considers that in all probability the *Plumbagella* embryo sac has been derived by reduction from the *Lilium* type. He found the latter present in all of the genera of Staticeae which he examined. The embryo sac of *Plumbago*, having eight nuclei produced by a megaspore mother cell nucleus, may then represent an intermediate stage in a reduction series leading to *Plumbagella*. The fusion of four polar nuclei in *Plumbago*, while having no counterpart in the *Lilium* type of embryo sac, may have arisen incidental to the failure of the synergids and antipodals to be organized. On the other hand, if the embryo sac of *Plumbago* be regarded as having been derived from a 16-nucleate sac of the kind found in the Penaeaceae (6), a possibility which I have already suggested, its relation to the type characteristic of the Staticeae would be rather remote.

Summary

1. The single ovule of *Plumbago capensis* is derived directly from the apical meristem of the floral axis, and thus is strictly cauline in origin.

2. The curvature of the ovule embraces a complete circle, so that the micropyle finally is directed upward.

3. Two integuments are present, but the outer one is not developed on the side of the ovule next to the funiculus.

4. Frequently the archesporium involves two cells, but as a rule only one is present.

5. The archesporial cell gives rise to a primary parietal cell and the megaspore mother cell. The former finally produces two layers of parietal tissue.

6. The megaspore mother cell develops directly into the embryo sac, no linear tetrad of megaspores being formed.

7. By the division of each of the four megaspore nuclei, which exhibit a crosslike arrangement, an 8-nucleate embryo sac is formed, the daughter nuclei remaining together in pairs.

8. From one of the micropylar pair of nuclei the egg is organized. No synergids are differentiated. Of the remaining seven nuclei, three usually degenerate, while four approach one another and soon fuse.

9. Occasionally one or more of the three nuclei which usually degenerate persist and form small naked cells which may later enlarge and become egglike.

10. The mature embryo sac consists normally only of a large egg, vacuolate above, and a primary endosperm nucleus formed by the fusion of the four polar nuclei.

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CHROMOSOME BEHAVIOR IN PINUS BANKSIANA FOLLOWING FERTILIZATION[1]

CONTRIBUTIONS FROM THE HULL BOTANICAL LABORATORY 453

J. M. BEAL

(WITH PLATES XIII, XIV)

Introduction

Although considerable work has been done on the development of the gametophytes, the process of gametic union, and the development of the embryo in gymnosperms, relatively little detailed attention has been given to the behavior of the maternal and paternal chromosomes during the divisions immediately following fertilization. While a number of investigators have described certain stages in the early embryonal divisions, these reports are in the main fragmentary and incidental to a more general study of other features. My observations are in such close agreement with those of certain investigators and so contrary to those of certain others, that it will not be amiss to review briefly a few of the reports.

In *Sequoia sempervirens* LAWSON (7) reported a longitudinal splitting of the long V-shaped chromosomes during the first embryonal division, and stated that the daughter halves as they pass to the poles are about half the size of the mother chromosomes from which they are derived. He observed about 32 chromosomes in the young sporophyte as compared with about 16 in the gametophyte. In *Bowenia* he (8) reported that in fertilization there is not a complete union of the gametic nuclei preceding the first embryonal division, but that each parental nucleus organizes a separate group of eight chromosomes. A spindle is developed for each group, and these remain separate not only in this first division, but during the four or five subsequent mitoses. This reported behavior appears to be unique in the plant kingdom.

BLACKMAN (1) found in *Pinus sylvestris* that the chromosomes in

[1] This investigation was aided in part by a grant to the University of Chicago from the Rockefeller Foundation.

the first division of the zygote fall roughly into two groups, each chromosome of which splits longitudinally during the first embryonal division.

In what is doubtless the most critical and thorough work yet done in the gymnosperms, FERGUSON (4, 5) described gametic union and early embryonal divisions in *Pinus strobus*. She observed the condensation of separate groups of chromosomes from the chromatin of each gametic nucleus following fertilization. These two groups of chromosomes remain separate and distinct while the multipolar polyarch spindles are being formed. The spindles then merge into a multipolar diarch spindle and the chromosomes become so arranged in the equatorial region of the spindle that the maternal and paternal elements can no longer be distinguished. She obtained one preparation in which a polar view of an equatorial plate stage showed 24 chromosomes (5, fig. 235). Twelve chromosomes were observed in the egg nucleus and it was estimated that the sperm brought in the same number. Her figures 236–238 all show clear indications of a longitudinal splitting of the chromosomes during the first embryonal division. In connection with figure 237 she states, "While this opening [the separation of the daughter chromosomes at the points of the spindle fiber attachments] is still inconspicuous, the two halves of a given chromosome become distinct throughout the entire length of the segment."

All the reports just cited agree that the maternal and paternal chromosomes split longitudinally during the first embryonal division, and that the diploid number is present as the result of gametic union of two haploid gametes.

In *Abies balsamea* HUTCHINSON (6) reported that following the fusion of the gametic nuclei two groups of chromosomes are formed separately in the prophases of the first division of the zygote, each group containing the haploid number of chromosomes. "As the two spindles unite the chromosomes become paired, at first approximating side by side, but soon twisting about one another and jointly looping into the form of a C." He states that this is a pairing and not a longitudinal splitting of the chromatin elements, and that the number of pairs is haploid. "Also, the twisting of the chromosomes about one another is identical with their behavior in what is generally re-

garded as a pairing during the prophase of the first reduction division." The bending into C- or V-shaped forms is followed by a transverse segmentation of each component of a pair at the angle of the bent chromosomes. These resulting segments, 4n in number, separate during the metaphases so that each daughter nucleus receives one-half of each of the paired elements. In the second division each of the half chromosomes splits longitudinally and separates as in a regular mitosis.

In *Stangeria paradoxa* CHAMBERLAIN (3) reported "the number of chromosomes, as counted at the equatorial plate stage of the first division of the fertilized egg, is 12; but later free nuclear divisions and also mitoses in root tips show that 24 is the diploid number; further, the mitosis at the formation of the ventral canal nucleus and egg shows 12 chromosomes, proving that this is the haploid number." He found four zygotes undergoing the first mitosis, all in the equatorial plate stage. "In all of these the number of chromosomes is 12, but the double character of the chromosomes is evident; and since 12 and 24 are the haploid and diploid numbers, I believe that there is a pairing of chromosomes at fertilization, as described by HUTCHINSON for *Abies*." No figures are given.

Among the angiosperms a single report of "pairing" between maternal and paternal chromosomes, followed by a transverse break during the first embryonal division, has been reported by WENIGER (9) for two species of *Lilium*. She gives three figures and one diagram, but her report is in disagreement with the findings of all other investigators for the angiosperms.

According to the chromosome theory of heredity, each chromosome carries a series of genes arranged in linear order. In fertilization two sets of chromosomes and hence two lots of genes are brought into association. Except for occasional and apparently rather rare upsets, the chromosomes contributed by the two gametic nuclei are maintained unchanged through equational division in the first embryonal as well as in all subsequent mitoses, the daughter chromosomes being distributed in equal numbers to the resulting daughter nuclei.

Because the type of division described in these three reports is so fundamentally at variance with the generally accepted ideas of

chromosome behavior and genetic theory, a reinvestigation of *Pinus* has been undertaken with the hope of clearing up some of the points at issue.

Material and methods

This report is based on a study of material of *Pinus banksiana* Lamb. The first collections were made in the spring of 1932 near Miller, Indiana, and fertilization was found to occur early in June. During 1933 additional fixations were made three times daily, from June 6 to 16 inclusive. Material was fixed at the time of collection, which was between 9:30 and 10:30 A.M. The carpellate scale was carefully pulled from the strobilus and the adaxial face of each ovule was sliced off so as to expose the megagametophyte directly to the fixing solution. The ovule was then cut or pulled from the scale and dropped quickly into the solution. Short pieces of branches bearing carpellate cones were taken from the trees, placed in a vasculum containing moistened paper towels, and brought into the laboratory, where fixations were made between 2:30 and 3:30 P.M. and again at 8:30 to 10:00 P.M. In every case the collections were made from more than one tree.

Several fixing solutions were employed, but the best results were obtained through the use of a modified Navashin's solution consisting of solution A (1 gm. chromic acid crystals, 7 cc. glacial acetic acid, and 92 cc. distilled water) and solution B (30 cc. formalin and 70 cc. distilled water). Equal volumes of the solutions were mixed just before using. Sections were cut at thicknesses ranging from 10 to 15 μ and stained in iron-alum haematoxylin.

Observations

First embryonal division

In agreement with Blackman (1), Ferguson (4, 5), Chamberlain (2), and other investigators it has been observed that the gametic nuclei of *Pinus banksiana* unite while they are both in the reticulate condition. Shortly afterward the reticula condense to form two groups of chromosomes which can be distinguished as having originated from the respective parental nuclei. A multipolar spindle then develops for each group, apparently independently at first, but soon the two spindles merge into a single multipolar diarch spindle

at the equator of which the chromosomes come to lie. The maternal and paternal chromosomes can now no longer be distinguished from one another (figs. 1 *a-d*). These four figures were taken from adjacent sections of a single nucleus which had been cut at a thickness of 10 μ and represent the equatorial plate stage during the first embryonal division. Several of the chromosomes have been cut in sectioning, but there are sufficient numbers of uncut ones to show the forms and structures characteristic of this stage. It is possible to count more than 20 chromosomes, and in all probability the number is 24, corresponding to the somatic or diploid number. None of the chromosomes gives indications of paired and twisted maternal and paternal elements. Further, the number of "fiber-attachment points" as shown by the drawings is approximately 24 instead of 12, thus confirming the presence of the diploid number of chromosomes at this stage. Some of the chromosomes have evidences of a longitudinal split.

Figures 2 and 3 are taken from adjacent sections of the same nucleus during a metaphase stage. They show the characteristic appearance of the chromosomes as observed in several nuclei during these stages. In agreement with FERGUSON'S (5) observations, the chromosomes at this stage show a separation of the daughter halves, not only at the fiber attachment points, but the two halves of a given longitudinally split chromosome are distinct throughout the entire length of each element. Although these two figures do not include the entire complement of chromosomes, more than 12 are present.

During early anaphase (fig. 4) the chromosomes are in the form of U's, narrow U's, and V's. An occasional one has one arm slightly shorter than the other, due to the fiber attachment point being submedian. No case of terminal or subterminal fiber attachment has been seen. More than 12 chromosomes are present in each group, counting the entire U or V as a single chromosome.

In a late anaphase stage (fig. 5) the form and appearance of individual chromosomes is essentially the same as in figure 4. In none of this material is there any evidence of a "pairing" of parental chromosomes nor is there any indication of a transverse segmentation at any of the stages in this division. The length of the chromosomes remains approximately constant throughout the process of

division, while the volume of the daughter halves as they pass to the poles appears to be about half that of the mother chromosome before it has split and separated in the metaphases.

Second embryonal division

In the second embryonal division the two daughter nuclei pass through essentially the same stages as did the nucleus of the zygote during the first embryonal division.

In the equatorial plate stage (fig. 6) the chromosomes have approximately the same general forms as in the corresponding stage in the first division, and they are of similar size in thickness and length. Only a portion of the whole complement is shown here, but counts made on this and adjacent sections indicate 24 chromosomes to be present, thus agreeing with the count determined for the preceding division.

During the metaphases (fig. 7) the chromosomes again have the same form and appearance as in similar stages of the first division. The manner of separation at the fiber attachment points and the indications of longitudinal splitting are practically identical in the two divisions.

Observations of the next two divisions have been made, but since they are so similar to corresponding stages in the preceding divisions it is unnecessary to figure or describe them.

Summary

1. During the first embryonal division in *Pinus banksiana* two groups of chromosomes and two spindles are developed. The spindles quickly merge into one multipolar diarch spindle, upon the equator of which the chromosomes come to lie in one group in which the maternal and paternal elements are no longer distinguishable from one another. Each chromosome splits longitudinally and the daughter halves pass to the poles, 24 chromosomes going to each pole. The second and subsequent embryonal divisions are similar to the first. There is no "pairing" between maternal and paternal chromosomes, nor any transverse segmentation during any of the divisions observed.

2. The only place where such interpretation seems possible is in the anaphases when the daughter chromosomes show the form of

U's and V's, so narrow or pointed at the place of attachment of the spindle fibers that the chromosomes may appear to have undergone or be about to undergo transverse segmentation. When these forms, however, are correlated with the entire series of observable stages which the chromosomes undergo during the first and subsequent embryonal divisions, this interpretation seems scarcely possible.

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EXPLANATION OF PLATES XIII, XIV

All the figures were drawn at table level with the aid of an Abbé camera lucida under a Zeiss apochromatic objective, 2 mm. N.A. 1.40, with compensating ocular 10×. Magnification is approximately 1600×.

PLATE XIII

FIG. 1 *a–d*.—Entire chromosome complement of an equatorial plate stage of first embryonal division. Fiber attachment points indicate the number of individual chromosomes present.

FIGS. 2, 3.—Metaphases from adjacent sections of the first embryonal division showing fiber attachment points and longitudinally split chromosomes.

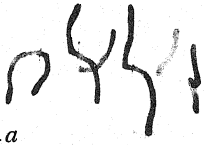
PLATE XIV

FIG. 4.—Somewhat early anaphase of first embryonal division showing typical forms of daughter chromosomes.

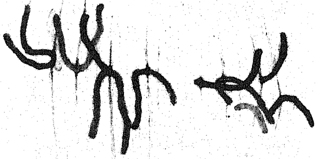
FIG. 5.—Late anaphase of first embryonal division.

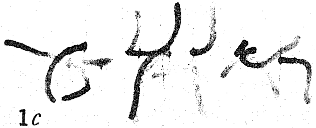
FIG. 6.—Equatorial plate stage of second embryonal division.

FIG. 7.—Metaphase of second embryonal division.

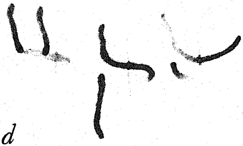


1*a*





1*c*



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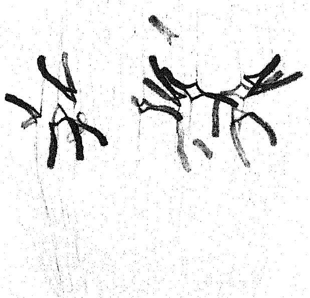

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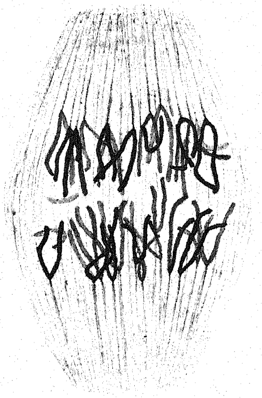
BEAL on CHROMOSOME BEHAVIOR



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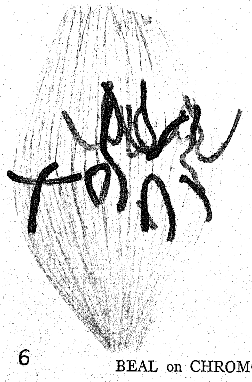

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BEAL on CHROMOSOME BEHAVIOR

ANATOMY OF THE XYLEM OF PSEUDOLARIX

ALAN S. PEIRCE

(WITH SIXTEEN FIGURES)

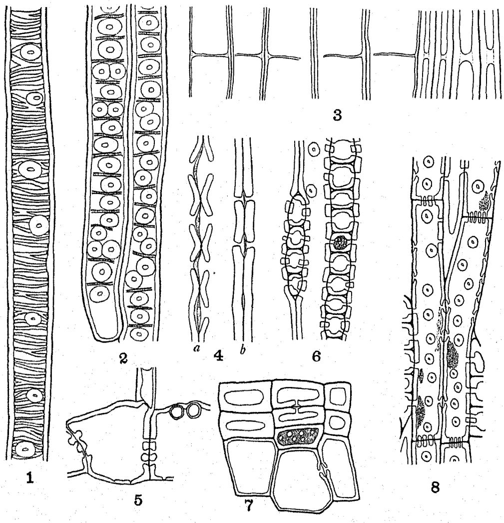
Introduction

So far as the writer is aware, the wood anatomy of *Pseudolarix* has been reported only once (3), and then rather superficially. JEFFREY, in his anatomical treatment of the Abietineae (9), bases his conclusions to a large extent on vascular anatomy and its phylogenetic significance. His main theses concern resin mechanism and ray tracheids. In works concerning the occurrence of single anatomical features in the Coniferales as a whole, GERRY (6) covers the distribution of the bars of Sanio, while ray tracheids are fully reported by HOLDEN (8). PENHALLOW (16) includes a more extensive anatomical study of six of the genera of Abietineae in his work on gymnosperms, although *Keteleeria*, *Cedrus*, and *Pseudolarix* are not considered. KANEHIRA (12, 13) in his comprehensive studies of Formosan and Japanese woods omits the genera *Cedrus* and *Pseudolarix*.

Along the lines of microscopic morphology, MIYAKE and YASUI (14) find the gametophytic development in *Pseudolarix* closely following the abietinean type. BUCHHOLZ (4) further observes the similarity in embryogeny of this genus to that of the Abietineae as a group.

The investigation reported here attempts to add to the knowledge of the wood anatomy of conifers as a whole and to supplement that of the already best known group, the Abietineae. The writer has undertaken to determine the many and diverse affinities that arise from microscopic anatomy and to integrate them into a logical phylogenetic relationship with other conifers.

The genus is monotypic and is indigenous to eastern China. The single species is known correctly as *Pseudolarix kaempferi* (Lindley) Gordon. A number of recent manuals include the morphological description of this genus, since it is cultivated at several plantings in the eastern United States.



FIGS. 1–8.—Fig. 1, closely spiraled metaxylem element showing rudimentary pits imbedded in spiral bands (radial). ×500. Fig. 2, bordered pits on tracheids of median spring wood, showing occasional biseriate arrangement (radial). ×240. Fig. 3, portion of trabecula passing from terminal wood to next year's growth (radial). ×240. Fig. 4, bordered pits in section contrasted: *a*, radial wall of spring wood; *b*, tangential wall of summer wood. ×500. Fig. 5, simple pit connection between marginal ray cell and final tracheid of summer wood (radial). ×500. Fig. 6, section through xylem rays in summer wood, showing connections with tracheid by means of simple pits (tangential). ×225. Fig. 7, end wall of wood parenchyma cell; note also bordered pit on tangential wall of late summer wood (transverse). ×225. Fig. 8, two entire wood parenchyma cells bordering rays and a tracheid by means of simple pits. Bordered pits connect these cells with each other and with following year's first spring tracheids; note also albuminous contents (tangential). ×225.

Investigation

General anatomical features

The vertical elements of *Pseudolarix* are wholly tracheidal, with the exception of terminal wood parenchyma. By this term is meant the occurrence of this cell type only in the last layer of summer wood, where it is abundant. The xylem rays are wholly parenchymatous.

The wood is white when cut and becomes darker on exposure. The heartwood becomes yellow-brown while the sapwood, which is 1–2 cm. in diameter, has a pale creamy shade. Specific gravity shows a figure of 0.534. The pith is approximately 0.5–1 mm. in diameter, in the outer layers of which nuclei are evident in stems as much as nine years old.

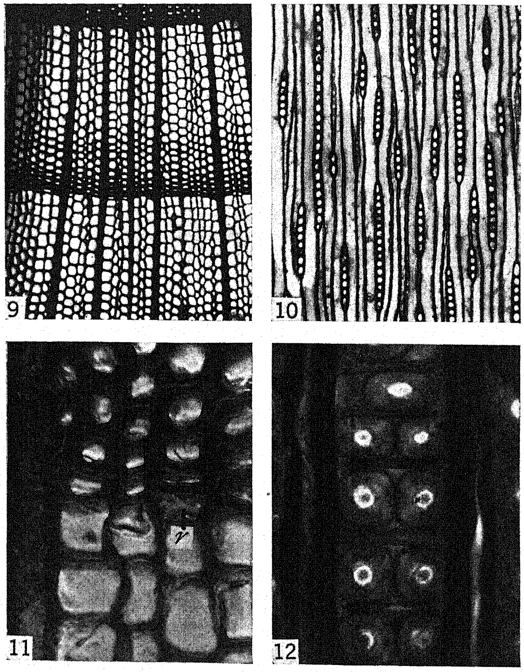
The transition from spring to summer wood is gradual, and shows no definite line of demarcation such as would be afforded by a girdle of resin cells or an abrupt thickening of tracheid walls. The extent of spring wood is from 20 to 30 cells, while that of summer wood is less, being from 8 to 18 cells. A distinct growth ring boundary is produced by the dense summer wood (fig. 9).

Stelar development is endarch, the condition typical of gymnosperms. The protoxylem is isolated, occurring at 15 to 20 points which project into the pith, and consists of thin-walled elements with spiral thickenings. The spirals are very loose in the first elements and become more compact, passing gradually into the type designated as metaxylem. These latter elements are closely spiraled, almost scalariform, and show an abundance of small rudimentary bordered pits imbedded in the bands (fig. 1). In the radial section passing through the greatest amount of protoxylem the extent is from three to five cells, while that of metaxylem is rather uniformly two or three cells.

Tracheids

Pseudolarix shows a moderate variation in tracheid length. The average is 2.5 mm., and a steady gradation includes the extremes of 1.8 and 3.4 mm. Table I gives the complete relative measurements of tracheidal parts.

Large and distinct bordered pits are uniformly abundant on radial walls, extending to the ends of cells (fig. 2). In uniseriate pitting the



FIGS. 9–12.—Fig. 9, transverse section; note gradual transition yet distinct growth ring boundary. $\times 80$. Fig. 10, tangential section; note height and straightness of uniformly uniseriate rays. $\times 80$. Fig. 11, transverse section showing resin cell plate (*r*) in terminal summer wood. $\times 480$. Fig. 12, radial section; note distinct horizontal crassulae and absence of vertical bars. $\times 800$.

horizontal diameters are slightly greater than the vertical, while in the biseriate arrangement, featuring the first four to six layers of spring wood, the compressed pits become somewhat squared (figs. 12, 13). In both instances the pit mouth is about one-third the diameter of the entire pit. The pits of summer wood are smaller and the mouth takes on a characteristic slitlike appearance (fig. 14). Smaller bordered pits are also found in moderate abundance on tangential walls in the last two to four layers of summer wood (fig. 8). In cross-

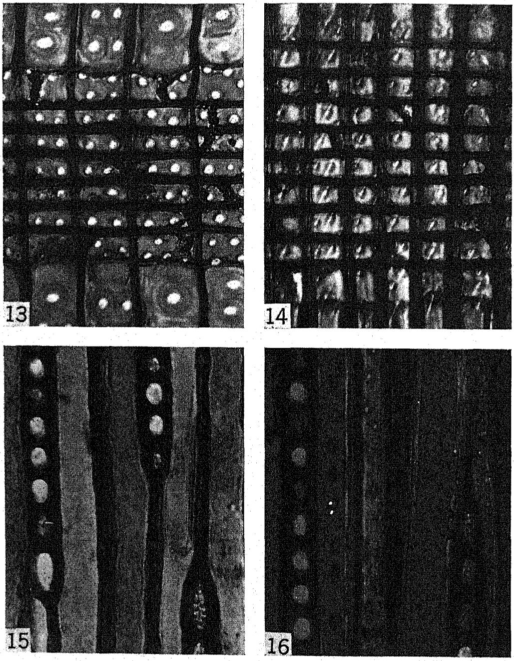
TABLE I

COMPARATIVE MEASUREMENTS OF PARTS OF TRACHEIDS, BASED ON FIFTY SAMPLES

	SPRING WOOD (μ)	SUMMER WOOD (μ)
TRACHEID		
Length	2547	2547
Radial depth	39.0	11.7
Tangential width	35.1	30.2
Wall, radial	1.9	3.4
Wall, tangential	1.5	4.8
BORDERED PITS		
Radial walls, mouth, horizontal	7.0	4.1
Radial walls, mouth, vertical	4.4	4.1
Radial walls, entire, horizontal	21.4	13.8
Radial walls, entire, vertical	16.8	13.8
Tangential walls, mouth	....	4.3
Tangential walls, entire	....	13.9

section these show a decided contrast to the usual pits on radial walls of spring wood (figs. 4, 7). Cracking of pit mouths is observed in the summer wood tracheid, taking the form of right-hand striations which often form a continuous spiral in the final layers. Tracheids are in direct communication, through pits, with ray cells in all regions of the wood (figs. 13, 14). In the final two or three layers the connections are of simple pits (figs. 5, 6).

Crassulae (**19**) occur consistently throughout the spring wood and are of sporadic occurrence in the summer wood. They are chiefly single and straight, although variations are seen occasionally (fig. 13). An indication of vertical bars between pits of biseriate arrangement is afforded under low power magnification. However, the oil immersion objective proves these lines to be an illusion, probably of



FIGS. 13–16.—Fig. 13, radial section of spring wood showing characteristic tracheid pitting and lateral ray pits. ×280. Fig. 14, same, in summer wood region. Fig. 15, tangential section of spring wood showing radial pits in section, and detail of rays. ×300. Fig. 16, same of summer wood showing wood parenchyma cells and communication with ray cells; note also bordered pits on tangential walls of parenchyma and tracheids alike.

refraction, while the horizontal bars stand out conspicuously (figs. 12, 13).

Trabeculae (**15**) are sometimes present and extend through the growth of several years (fig. 3). These structures, apparently homogeneous with the wall of the element through which they pass, presumably arise at the time of the formation of each tracheid of the series by the cambium.

Hexagonal, and rarely rectangular, crystals have been occasionally observed in various positions in summer tracheids. They are usually longer than the tangential width of the element and cause a bulging when they lie horizontally.

Wood parenchyma

The wood parenchyma in *Pseudolarix* is strictly terminal, occupying almost the entire final layer of summer wood. The cells show a great variation in length, from 40 to 250 μ, and average roughly 150 μ. Their depth and width are fairly uniform, the former varying but slightly from 15 μ and the latter constant at 30–33 μ. These cells thus show a greater depth than summer tracheids, are approximately as wide, and are much shorter.

The longitudinal walls are slightly thinner than those of summer tracheids, while the invariably horizontal end walls are thick and coarse, marked with two to six or more simple pits (figs. 7, 8, 11, 16). Pitting on radial walls is variable; communication with ray cells is by means of simple pits, a feature striking in its clarity; bordered pits occur between adjacent parenchyma cells; while between parenchyma cell and tracheid the pits are simple (fig. 8). Bordered pits occur abundantly on the tangential walls (figs. 8, 16).

In stained sections wood parenchyma cells contain a granular substance, sometimes extensively distributed, sometimes drawn up in semblance of nuclei (fig. 8). The alkannin test indicates the presence of resin in sections prepared from dried material.

Xylem rays

Ray tracheids are not present in the normal wood of *Pseudolarix*, nor have any been detected in wounded tissues. Several facts combine to indicate that rays are, except in possibly unobserved in-

stances, wholly parenchymatous. These are: the presence of nuclei indiscriminately in both marginal and intervening cells; the similarity of cells in all regions of the ray; and the absence of dentations or ridges on transverse walls. Rays vary from one to twenty-eight cells in height, averaging between eight and nine, while the majority contain five to ten cells. They are without exception uniseriate and are straight tangentially (figs. 10, 15). Several instances of ray fusion have been observed, occurring more often as traumatic responses than as a normal condition. Such fusions are marked by great irregu-

TABLE II

MEASUREMENTS OF RAY CONSTITUENTS

	SPRING WOOD (μ)	SUMMER WOOD (μ)
End walls	2.2	3.0
Transverse walls	1.9	2.4
Radial walls	2.3	2.4
End wall pits	3.0	3.0
Transverse wall pits	4.0	3.5
Radial wall pits, mouth	5.9	3.0
Radial wall pits, entire	7.8	4.2

larities in cell shape. No splitting or divergence of rays already formed by fusion has been observed.

Wall structure of ray cells is uniform. The end walls are coarse, thick, and profusely marked with simple pits (fig. 13). Transverse walls are smooth, thinner, and broken sparingly by simple pits. Radial walls are also smooth and thin, with narrowly bordered "piciform" (2) pits occurring two to four to a crossfield in the spring wood and one to two in the summer wood, the number being governed apparently by the size of the crossfield (figs. 13, 14). Transverse walls, moreover, are straight in the spring wood but converge slightly at the ends in the summer wood, giving the cells a convex shape. End walls occur in a great variety of positions in spring wood, while in summer wood they are more consistently vertical.

Measurements of ray cells reveal a difference of length in spring and summer wood, while the height is constant, 21–25 μ. Length in spring wood gives the maximum figure of 200 μ and average figure

of 180 μ, while in summer wood the minimum is 120 μ and the average 140 μ. Marginal cells are slightly variable in height, the distal wall showing gradual undulation. Table II gives the measurements of ray cell walls and pits.

Discussion

The type of tracheid pitting, in its clarity and abundance, shows similarity to the Taxodineae in general and to the majority of the Abietineae, with the exception of the few species of *Pinus* which lack this feature altogether (13). The form and distribution of crassulae show strong affinity to both the Abietineae and the Taxodineae (6).

The absence of resin passages constitutes a condition typical of JEFFREY'S subtribe Abieteae (9), one genus of which, *Abies*, may be designated as transitory since these structures occur in at least three of its species: *A. nobilis* (16), *A. firma* (13), and *A. venusta* (18). Fusiform rays, rays containing horizontal resin passages, are produced traumatically in *Cedrus* (11), the accepted primitive genus of Abieteae. These structures are not reported for any of the other genera, including the material of *Pseudolarix* observed by the writer.

The terminal distribution of wood parenchyma in *Pseudolarix* is duplicated, in the Abieteae, by *Tsuga* (16) and *Cedrus* (11), and in the Pineae by *Picea*, *Pseudotsuga*, and *Larix* (11). However, JEFFREY further asserts that *Tsuga* has a tendency toward diffuse distribution. In the matter of wood parenchyma distribution, therefore, *Pseudolarix* is grouped with *Cedrus* as showing the primitive type. In the entire Taxodineae the distribution of wood parenchyma is either diffuse or girdled in early summer wood, but never strictly terminal.

Pseudolarix is the only genus of the Abietineae without ray tracheids, and thus is similar to the Taxodineae, where they are rarely found (10, 7). The writer agrees with HOLDEN, who was unable to find ray tracheids, even in severely wounded branches. This genus appears to be little affected by wounding, however, as HOLDEN states further that the formation of traumatic resin passages is much less marked here than in other genera of the Abietineae. The fact that the writer also was unable to find these structures as a traumatic response serves to uphold this statement.

Ray cell pitting shows a great similarity to the Abietineae with the exception of most species of *Pinus* (1, 2, 5, 11). Moreover, the shape and occurrence of these pits strongly suggest the type found in *Sequoia* (7, 16) and *Cunninghamia* (10, 13), but the wide variation shown in the remaining genera of the Taxodineae is not present in *Pseudolarix*. BAILEY (2) reports great variation in ray lateral pitting in the hard and soft pines alike, and regards the piciform type as primitive. The nut pines exhibit the latter type and compare favorably with Cretaceous specimens. Since the majority of the Abietineae, however, and even of conifers in general, have distinctly piciform pits, we may confine the evolutionary sequence there described to the genus *Pinus*. As the ray structure of *Cedrus* stands midway between the Pineae and the Abieteae (5), so does that of *Pseudolarix* appear to be transitional between the Abietineae and the Taxodineae.

The spiral sculpture observed in summer wood tracheids of *Pseudolarix* is similar to that reported for *Pinus*, *Picea*, *Larix*, and *Pseudotsuga* (1), and *Keteleeria* and *Tsuga* (12). This feature is almost entirely foreign to the tribes Taxodineae and Cupressineae (13), and the spirals of *Taxus* and *Torreya* are easily recognizable as structurally different. Spirals therefore serve to unite the genera of the Abietineae without apparently exhibiting an evolutionary sequence.

Summary

The anatomical characters reveal that *Pseudolarix* occupies a relatively high position in the Abietineae. The anatomy, moreover, merges with that of the Taxodineae in a number of features, indicating a general transition in this direction.

The writer is indebted to Dr. JOHN T. BUCHHOLZ for furnishing the material, collected by him on Long Island, New York, and for his valuable suggestions and criticisms of the work.

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ELECTROPHORESIS OF LATEX AND CHROMOSOME NUMBERS OF POINSETTIAS

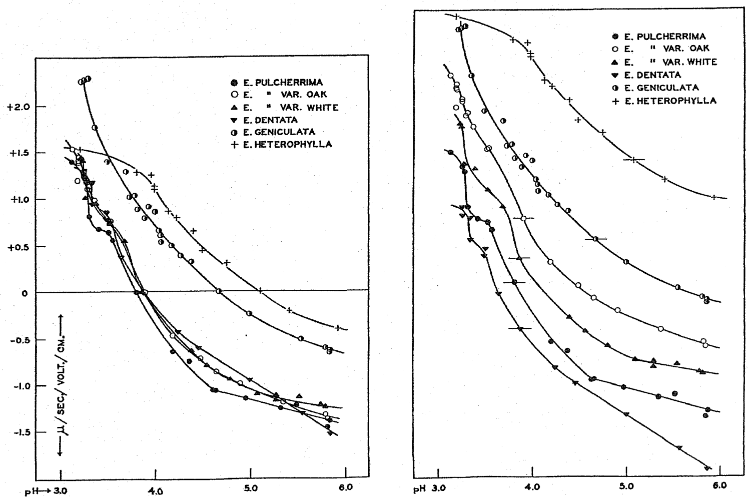
LAURENCE S. MOYER

(WITH NINE FIGURES)

In a recent paper (10) the writer presented determinations of the isoelectric points of the latex particles from more than twenty species of *Euphorbia*. Each latex was suspended in a series of M/50 acetate buffers and its electrophoretic mobility determined in a modified Northrup-Kunitz microelectrophoresis apparatus. The curves of mobility (in μ/sec./volt./cm.) plotted against pH proved to be constant for each of the species investigated. These curves (including isoelectric points) can be arranged into several different groups whose members have similar shapes. With few exceptions the groups agree with the taxonomic relationships already established for the genus.

The most noticeable exception occurred in the section Poinsettia. In this subdivision all of the investigated species are taxonomically very closely related (3). As may be seen in figure 1, *Euphorbia dentata* Mich. and *E. pulcherrima* Willd., with its two varietal forms, white and oak, are very similar in respect to shape of curve and isoelectric points (i.p.) In figure 2 the curves are plotted on the same scale but spread apart to show this similarity in shape. *E. heterophylla* L., another member of the group, however, shows no close relationship to the other species in either isoelectric point or in curve shape.

The writer has recently investigated the electrophoretic behavior of the latex from *E. geniculata* Orteg., another member of the section, in addition to the species whose curves are shown. This species was grown from seed generously supplied by the Muséum National d'Histoire Naturelle, Paris. A detailed description of methods employed is given in an earlier paper (10). The curve of mobility has been drawn to the same scale as the other curves, and is included in figures 1 and 2. As in the previous work (10), all points are corrected for temperature and are calculated to 25° C. The latex was tapped



FIGS. 1–2.—Fig. 1, mobility curves of latex particles from the section Poinsettia; all species plotted to same scale. In fig. 2 (on right), curves are spread out to show shapes.

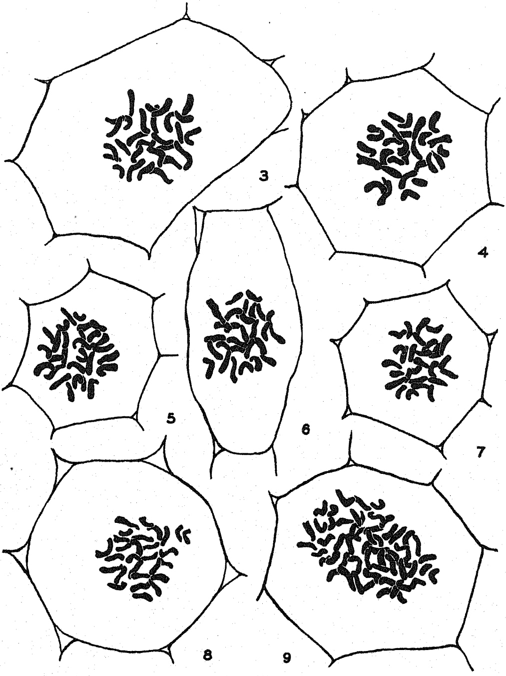
from four healthy specimens in order to eliminate individual variations as much as possible.

The curve is smooth and most like that of *E. pulcherrima* var. oak. The isoelectric points of the two curves, however, are widely divergent. The latex particles of *E. geniculata* do not move in an electric field at pH 4.7, while those from *E. pulcherrima* var. oak are isoelectric at pH 3.9. The curve for *E. heterophylla* is no more closely related by its shape to *E. geniculata* than to the members of the *E. dentata-E. pulcherrima* group.

Since the position of the point at pH 3.2 on the *E. heterophylla* curve caused most of the difference in shape between it and the curve for *E. geniculata*, this point was checked again and found to have the same position (within experimental error) as reported previously (10). The i.p. of particles of *E. heterophylla* latex was redetermined with plants grown from different seed and was again found at pH 5.1. This is not very close to that of *E. geniculata*. Since neither the systematic position nor the geographical distribution of these species offers a clear explanation for this divergence, it was thought that an investigation of chromosome numbers might be of value.

CARANO (4) reported that *Poinsettia pulcherrima* R. Grah. (= *E. pulcherrima* Willd.) has 10 chromosomes in the haploid cells "con sufficiente approssimazione." The chromosomes of the other species and varieties in this section apparently have never been counted.

Root tips were fixed in Nawaschin's fluid as modified by SAX (14) and dehydrated and imbedded in paraffin by means of ZIRKLE's n-butyl alcohol technique (17). Transverse sections were cut 10 μ thick, stained in crystal violet, and destained by NEWTON'S iodine method (11). A 15× orthoscopic ocular, a Zeiss HI 100 objective, n.a. 1.30, and a camera lucida were used in counting and drawing. ZETTNOW'S solution of copper sulphate and potassium bichromate diluted to one-third was used as a light filter. This solution gives nearly monochromatic light and makes objects stained violet appear as black. The results of the counts are given in table I while figures 3-9 show drawings of typical somatic metaphase plates. The table also presents data for a hybrid, *E. pulcherrima* var. oak × *E. pulcherrima* var. white, obtained from the Missouri Botanical Gar-



FIGS. 3–9.—Somatic metaphase plates of poinsettias: fig. 3, *Euphorbia pulcherrima* var. white, 2n = 28; fig. 4, *E. pulcherrima* var. oak, 2n = 28; fig. 5, *E. pulcherrima* var. oak × white, 2n = 28; fig. 6, *E. pulcherrima*, 2n = 28; fig. 7, *E. geniculata*, 2n = 28; fig. 8, *E. dentata*, 2n = 28; fig. 9, *E. heterophylla*, 2n = 56. × 2000.

den (2). This hybrid has pink bracts and leaves similar to the oak variety. No electrophoretic curve is given for this cross.

All of the species investigated in this group which have similar surfaces on their latex particles, as shown by the resemblance of the curves and the position of the isoelectric points (table I), have also like chromosome numbers. Although the curve of *E. geniculata* is somewhat divergent, it likewise has a diploid number of 28. On the other hand, *E. heterophylla* appears to be tetraploid with a somatic complement of 56 chromosomes, and it is therefore genetically very different from the other species.

TABLE I

CHROMOSOME NUMBERS AND ISOELECTRIC POINTS OF POINSETTIAS

Species	Latex isoelectric point (pH)	Chromosome number (2n)
E. dentata	3.9	28
E. pulcherrima	3.8	28
E. pulcherrima var. white	3.9	28
E. pulcherrima var. oak	3.9	28
E. pulcherrima var. white×oak	3.7	28
E. geniculata	4.7	28
E. heterophylla	5.1	56

In this connection it is interesting to note that ZADE, by the immunological reactions of the seed proteins (16), was able to separate the various species of *Triticum* and *Avena* into groups which are identical with those already formulated on a taxonomic basis. In a study of the chromosomes of *Triticum*, SAKAMURA (12) and later SAX (13) reported that each group has different chromosome numbers. The haploid numbers are as follows: einkorn group = 7, emmer group = 14, and spelt group = 21. KIHARA (7) likewise showed the same type of relationship between the chromosome numbers of different species of *Avena* and the groups proposed by ZADE. In a chemical study of the purified prolamines of corns and wheats, HOFFMAN and GORTNER (6) were unable to show any essential differences between the prolamines from the three wheat groups but were able to differentiate the wheat and corn groups. Their work was

confirmed by LEWIS and WELLS (8), who investigated the anaphylactic reactions of these proteins. Hence it is obvious that the mixed seed proteins (which presumably included the nucleoproteins) of wheat show differences which correspond to natural relationships and which are not shown by the purified prolamines.

Chromosome numbers of different members of the Centrospermae have also been shown by TISCHLER (15) to be correlated with the taxonomic groups proposed by MEZ and ZIEGENSPECK (9) on the basis of their serological reactions.

FUKUSHIMA and MARUYAMA (5) have recently investigated the immunological relationships and chromosome numbers of eight species of *Brassica*, and they find that the species fall into four groups. The members of each group have the same chromosome number and protein relationships, yet they are noticeably different from the members of any of the other three groups.

In the case of the latex particles of poinsettias, which are coated at least in part with proteins, the electrophoretic differences may not be due to a specific difference in the chemical constitution of the proteins themselves but to a difference in the proportions of the several proteins adsorbed on the surfaces. The composition of this hypothetical mixture appears to be constant in each species and to differ little among individual plants, but the proportions are evidently different in different species. When the curves are arranged in order of decreasing i.p., as noted before, they become less smooth as we pass down the pH scale (fig. 2), until the smooth even curve of *E. geniculata* is reached. The shape of this curve is almost identical with those of the curves obtained for pure proteins (1). Possibly the curves of the other members of the 28-chromosome group are conditioned by a mixture on the surfaces of the particles which is composed of protein in decreasing amounts plus another ampholyte of low i.p. If more species were investigated, a complete series might be found extending from *E. dentata* to *E. geniculata*. It should be noted, however, that the isoelectric points when plotted on the usual pH scale show much less divergence than when the actual concentrations of hydrogen ions are expressed. The difference between the i.p. of *E. pulcherrima* and that of *E. geniculata* is then ten times the difference between that of *E. geniculata* and *E. heterophylla*.

Both the electrophoretic behavior of these latices and their chromosome numbers appear to divide the species into three groups. The first group, composed of *E. pulcherrima* with its varieties and *E. dentata*, is linked to some extent to *E. geniculata* by an identical chromosome number (not necessarily an indicator of close relationship, to be sure) and possibly by its curve shape, but it differs in the position of the i.p. *E. heterophylla*, with 56 chromosomes and with both a different curve shape and a different i.p., does not seem clearly linked to either of the other groups.

Summary

1. The electrophoretic mobility curve for the latex particles of *Euphorbia geniculata* Orteg. has been determined in a modified Northrup-Kunitz apparatus.

2. The curve of *E. geniculata* latex is definitely different in shape and in the position of the isoelectric point from *E. heterophylla*, but, although its isoelectric point differs from those of *E. dentata* and of *E. pulcherrima* and its varieties (which lie close together), it seems related to them in shape.

3. The chromosomes from the root tip cells of four species and three varieties of the genus *Euphorbia*, section Poinsettia, have been counted. *E. dentata* Mich., *E. pulcherrima* Willd., *E. pulcherrima* var. oak, *E. pulcherrima* var. white, *E. pulcherrima* var. white $\times$ *E. pulcherrima* var. oak, and *E. geniculata* Orteg. are all characterized by a diploid chromosome number of 28, while *E. heterophylla* L. is tetraploid with a somatic number of 56.

4. These chromosome numbers are correlated with the grouping determined by the electrophoretic mobility curves and isoelectric points of latex particles found for the section.

The writer is indebted to Professor Conway Zirkle for valuable advice and assistance.

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ORIGIN OF ADVENTITIOUS ROOTS IN COTONEASTER DAMMERI

FLORENCE WOLFE

(WITH NINE FIGURES)

Introduction

In general, the term adventitious refers to any organ produced on the body of a plant in an abnormal position. Shoots produced from roots, or roots from aerial stems and leaves, are examples of this type of organ. Aerial stems of many species of plants show a tendency to produce adventitious roots, especially when they come in close contact with the soil. These roots may appear at any place on the stem, or in special positions, such as in the axils of buds or below the buds. Their origin is dependent upon the continued organized activity of one or more meristematic cells, or upon the renewal of meristematic activity of some other cell or group of cells. The present study was made to determine the origin of the adventitious roots on the stem of *Cotoneaster dammeri* Schneid. (*C. humifusa* Duthie) (1).

A review of the literature indicates that most of the investigation dealing with adventitious roots on stems is comparatively recent. CORBETT (4), in working with both woody and herbaceous cuttings, states that "roots take their origin in and are an extension of the active, formative material of the cambium." He maintains that the place of origin in cuttings of hardwoods is nearer the true cambium than it is in herbaceous cuttings of *Geranium*, *Coleus*, and allied plants. VAN DER LEK (5) states that roots of cuttings of *Salix*, *Populus*, and *Ribes nigrum* originate from preformed "root germs." These he reports to be closely associated with the cambium and located at the end of a medullary ray. WILSON (11) finds that epidermal and cortical cells in the axil of a branch bud of *Roripa austriaca* divide and give rise to the new root. SMITH's investigations (8) show that the root initial in cuttings of *Clematis* are derived from the fascicular cambium, and that the earliest embryonic stages extend from the cambium layer into a medullary ray. TAYLOR (9), in his

experiments with cuttings of *Acanthus montanus*, concludes that the meristems of roots originate from the cambium. PRIESTLEY and SWINGLE (7) find that roots which arise upon the young stems of *Veronica beccabunga* are pericyclic in position. Furthermore, they believe that the roots are not derived from one cell, but "are a result of the organization of a group of cells bordering upon the vascular cambium which have remained or again become meristematic." LEMAIRE (6) and VAN TIEGHEM and DOULIOT (10) contend that root primordia of adventitious roots on stems originate from the pericycle. CONNARD and ZIMMERMAN (3) find that adventitious roots from cuttings of *Portulaca oleracea* arise in the medullary rays from the interfascicular cambium. CARLSON (2), in work on *Coleus blumei*, states that "adventitious roots arising between the vascular bundles from the bases of young cuttings originate in one to several adjacent cells of the pericycle."

With the exception of the one case just mentioned in which the adventitious roots originated from the cortical and epidermal cells, all previous research indicates that these roots originate from cells of either the cambium or pericycle.

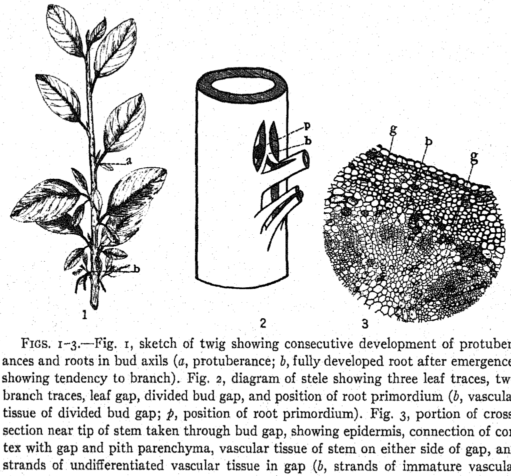
There are about forty species of *Cotoneaster* growing in the temperate regions of Europe, Asia, and North Africa. They are woody plants of the family Rosaceae, subfamily Pomeae. *Cotoneaster dammeri*, or *C. humifusa* as it is sometimes called, was introduced into the United States a quarter of a century ago from the mountains of China. It is an evergreen prostrate form, and is grown chiefly for landscaping purposes because of its showy fruits (12). As it creeps over the ground, it readily gives rise to a single root in the axil of each bud.

Investigation

Several plants of this species were obtained in September from a nursery and placed in a greenhouse for the winter. During the fall and early winter months, sections of the stem about 1 cm. in length were cut from the nodes, fixed in formalin-acetic-alcohol, and imbedded in paraffin. Cross and longitudinal sections, 10 μ in thickness, were made, mounted in series on slides, and stained with safranin and gentian violet.

At first a single small protuberance appears in the axil of each

bud, beginning at the third to sixth nodes from the tip of the growing stem (fig. 1 *a*). The protuberances become larger and larger in descending order to the tenth to fifteenth nodes, where fully formed roots are present. They emerge perpendicularly to the long axis of the stem at one side of the protuberance. The long axis of each root forms a 30° angle, with a radial line drawn from the center of the stem through the center of the long axis of the bud. The fact that these roots appear at such regular positions leads one to conclude



FIGS. 1-3.—Fig. 1, sketch of twig showing consecutive development of protuberances and roots in bud axils (*a*, protuberance; *b*, fully developed root after emergence, showing tendency to branch). Fig. 2, diagram of stele showing three leaf traces, two branch traces, leaf gap, divided bud gap, and position of root primordium (*b*, vascular tissue of divided bud gap; *p*, position of root primordium). Fig. 3, portion of cross-section near tip of stem taken through bud gap, showing epidermis, connection of cortex with gap and pith parenchyma, vascular tissue of stem on either side of gap, and strands of undifferentiated vascular tissue in gap (*b*, strands of immature vascular tissue; *g*, parenchyma of gap).

that a cell or a group of cells in a specific position gives rise to the root primordium.

The structure of *Cotoneaster dammeri* does not differ greatly from that of most woody plants. The very young stem shows a dictyostelic arrangement of the vascular tissues with collateral bundles. However, this is not the case in older portions of the twig. In sections beginning with the fourth node from the tip the vascular

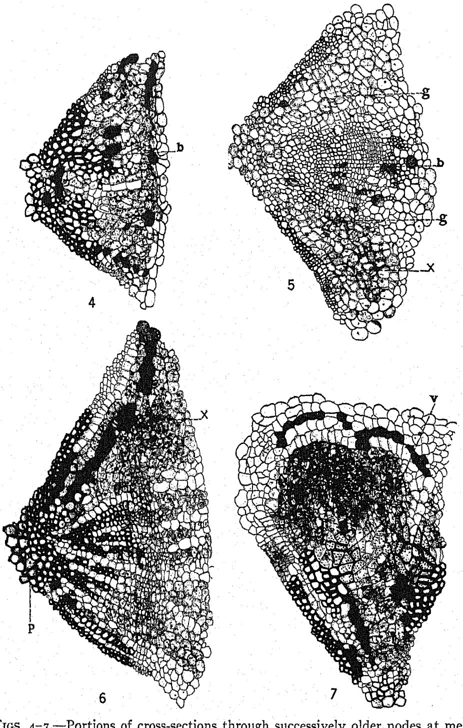
strands are connected laterally by the lateral extensions of the first formed ones, thus forming a continuous cylinder. Three vascular bundles arising from the stele supply the leaves and constitute the leaf trace. These bundles pass out into the petiole of the leaf at a sharp angle from the stem and unite very early to form a single bundle within the petiole. Figure 2 shows the stele with leaf trace.

The leaf gap is narrow and extends but a short distance above the point where the traces leave the vascular cylinder. This gap does not close, but merges with the branch gap, as will be shown later.

Two vascular bundles, the branch traces, arise from the vascular ring on either side of the leaf gap and unite within a short distance to form a complete stele in the young branch. The gap left in the vascular ring of the stem by the outward growth of these branch traces is continuous with that of the leaf gap (fig. 2), although it is slightly greater in width. The leaf and branch gaps are both composed of parenchyma and extend from the pith to the cells of the cortex.

The branch gap extends longitudinally to a distance equal to the length of a fully formed bud. It increases in width for a short distance and then gradually decreases until it becomes entirely closed. Generally a few strands of vascular tissue arise from the branch traces as they approach the outer portion of the stem (fig. 2 *b*). These strands pass up through the branch gap and in most cases unite to form a small irregular bundle in the upper central portion (figs. 2 *b*, 3 *b*, 4 *b*, 5 *b*). Figures 4, 5, and 6 are portions of a cross-section of the stem at the branch gap through the median line of development of the root primordium, and include only the tissues of the branch gap extending from the pith to the cortex, with a small number of cells of the vascular tissue of the stele on either side. The maturation of the vascular tissue in the gap is not completed so soon as that of the vascular ring (fig. 3 *b*). This last section is one taken from a branch gap near the tip of the stem and includes the cortex and epidermis beyond the branch gap.

Sometimes the union of the scattered strands does not always occur (fig. 3 *b*). In most cases, however, the parenchyma of the gap becomes divided into two parts, occupying a position on either side of the central strand of vascular tissue (fig. 5 *g*). The cells of this parenchyma are thin walled and have large vacuoles.



FIGS. 4–7.—Portions of cross-sections through successively older nodes at median line of development of root primordia. Fig. 4, very early stage in formation of root primordium with two vascular strands in gap. Fig. 5, older stage of root development showing recent divisions of cells with single vascular strand in center of gap. No development of cells can be detected on other side of gap in root formation (*g*, parenchyma of gap; *b*, vascular tissue; *x*, early development of root primordium). Fig. 6, later stage of primordial development than in fig. 5, showing elongation of cells between center of meristematic activity and vascular tissue (*x*, elongating cells at base of meristematic group). Fig. 7, one half of gap on side of root development, showing (left) still older root primordium with tracheids connecting with vascular cylinder of stem and (right) vascular tissue of gap (*v*, tracheids of root connecting with vascular strands of stem).

The outer layers of pith, when mature, consist of small cells that are much thicker walled than those of the branch gap (fig. 6*p*).

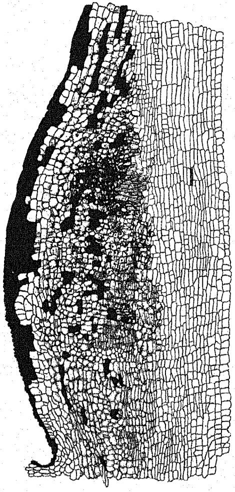
The epidermal cells become heavily cutinized, and the outer cells of the cortex develop into chlorenchyma at an early stage. No endodermis is present, as is generally the case in most woody stems of angiosperms. The pericycle varies in thickness from three to five cells, and in many cases is very difficult to distinguish as it merges into the thin walled cells of the inner cortex. In older, well developed stems, pericyclic fibers are prominent in scattered groups around the outside of the primary phloem (fig. 9*f*). This figure is a section of the stem through a fully developed root. The amount and development of secondary xylem and phloem depend entirely upon the age of the portion of the stem in question, and need no further discussion at this time. Scattered cells in various parts of the stem are filled with tannin and appear very dark after staining.

For the study of very early stages, serial sections were examined from the first three nodes at the top of the stem. The parenchymatous tissue of the gap was well divided into two groups, one on either side of the group of immature vascular tissue (fig. 3*g*). None of the cells in the divided gap revealed any indication of meristematic activity. The thin walled cells with their inconspicuous nuclei gave evidence that no unusual activity was taking place.

Figure 4 represents a section similar to the preceding one, but made from a slightly older node. Cell division is evident on one side of the gap near the cortex, owing to the appearance of abundant cytoplasmic contents and large nuclei in the cells. The activity of these cells is different from that of a similar group on the opposite side of the gap, or from those in a like position in figure 3.

In sections of slightly older nodes the meristematic region increases in size (fig. 5*x*), owing to the division of the original cells of the group and the reactivation of surrounding parenchyma cells. In figure 5 approximately 15 cells in one plane are involved, with evidence of at least two very recent divisions near the center of the group. In older nodes greater activity is apparent (fig. 6*x*). Many of the cells near the center of the active group show recent division, occupying about the same amount of space as the mother cells from which they arose. At the base of the group some of the cells have elongated.

These later develop into vascular tissue. Figure 7 shows the mass of active tissue becoming rounded, with the elongated cells at the base developing into vascular tissue which connects with the vascular elements of the stem on one side and with that of the center of the gap on the other.



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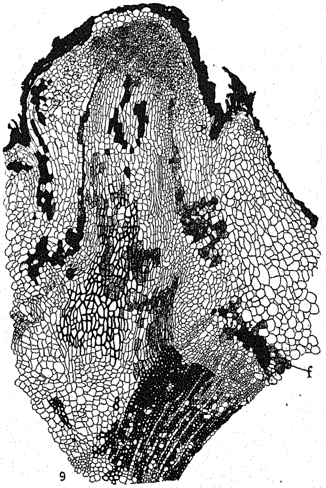

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FIGS. 8, 9.—Fig. 8, longitudinal section through protuberance, showing primordium in approximately same stage as in fig. 6. Cells at base of root show differentiation into tracheids; cells of cortex show presence of tannin in darkly stained areas, and at lower left a portion of upper part of bud is shown. Fig. 9, fully differentiated root breaking through cortex and epidermis with vascular tissue connecting with vascular cylinder of stem.

The structure of the root primordium and its position relative to the gap may be better understood from a longitudinal section cut in direct line, with the parenchyma on one side of the bud gap because of the vertical orientation of the parts. Figure 8 illustrates such a

section in about the same stage of development as in figure 7. The small projection at the lower left shows the point of attachment of the upper portion of the bud. The cortex is continuous with the parenchyma of the gap and finally merges into the central pith region at the right. The formation of the protuberance out of which the young root emerges is due, not only to the formation of the root primordium, but also to the division of some of the cells in the cortex. As shown in this section the root originates slightly above the center of the protuberance. The black body in figure 2 illustrates its position with reference to the stele.

From this time on development is rapid if conditions are favorable, that is, if the stem is in close contact with the soil. If such is not the case the root remains in a state of dormancy inside the protuberance until conditions are favorable for renewal of activity. Differentiation into the various root tissues occurs as the root forces its way outward. It increases rapidly in diameter, while the cells back of the tip elongate and form the cortex and central cylinder. The connection of the vascular tissue of the root and stem is completed (fig. 9) and the endodermis becomes clearly distinguished. The root apparently forces its way through the cortex and epidermis by pressure, although some chemical dissolution may take place. In many cases the root branches after attaining a length of half an inch (fig. 1 *b*).

The unusual feature in the entire development is that the root primordium originates on one side of the gap only. Although the parenchyma tissue on both sides of the branch gap appears identical in the early growth of the stem, no development occurs except on one side. This is due to resumption of the activity of these cells.

Summary

1. Adventitious roots of *Cotoneaster dammeri* arise from only one of the two groups of parenchymatous cells in the divided bud gap.

2. Formation of the root primordium is apparently due to the resumption of activity of the parenchyma cells.

3. The cortical cells in the region of the bud axis produce a slight protuberance through which the young root emerges.

4. Vascular elements of the young root arise very early.

5. The root is fully differentiated into the cortex and central cylinder by the time that it is ready to emerge.

6. Development of root initials occurs consecutively as the stem elongates.

The writer wishes to express her gratitude to Dr. M. C. CARLSON, of Northwestern University, for her kind assistance and criticisms during the progress of this work.

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NEW OR INTERESTING MYXOPHYCEAE FROM MISSOURI

FRANCIS DROUET

(WITH TWELVE FIGURES)

A few new or otherwise interesting blue-green algae have appeared in my collections from time to time during the past five years. The recent discovery of a number of unidentified specimens in the Herbarium of the Missouri Botanical Garden, collected by Dr. WILLIAM TRELEASE, brought to light several species in this group which have not previously appeared in the lists of Missouri algae (2, 3, 5, 6). *Scytonema alatum* (Carm.) Borzi has lately been recorded from Shannon Co. by SHARP (8).

The Myxophyceae are a difficult group, not only because of their few and minute taxonomic characters, but also because these characters may be easily changed by the mode of preservation. Formalin and other fixing agents produce granules and distortions of shape not found in living material. Dried algae are not always satisfactory for study. Living material cannot always be conveniently examined, especially on collecting trips of more than a day's duration, since deterioration sets in shortly after collection. The most satisfactory method of fixing which I have found is with a 3–5% aqueous solution of commercial formalin (40% formaldehyde). Plenty of liquid should be used so that the killing agent may penetrate throughout the mass as quickly as possible. Most of the observations on fixed material have been supplemented by the examination of living material.

BOYE PETERSEN (1) and GEITLER (4) have discussed the relative merits of diagnostic characters among the blue-green algae, and I have here placed most emphasis upon size and shape of cells, especially those at the ends of the trichomes, and the reaction of chlor-zinc-iodine upon the sheaths. Chlor-zinc-iodine[1] solution is prepared

[1] The latin equivalent of the name of the reagent, chlor-zinc-iodine, as employed by GOMONT, is *chlorozincicum iodurátum*, which, when translated, means "iodinized chlor-zinc."

according to the formula given by NOWOPOKROWSKY (7), here translated as follows:

> Dissolve 20 gm. of $ZnCl_2$ in 8.5 cc. of water with the aid of heat. Cool and add, drop by drop, the following solution until, after shaking, the iodine which is precipitated fails to dissolve further: 3 gm. of KI, 1.5 gm. of I_2, and 60 cc. of water. About 1.5 cc. of the latter solution is necessary.

MYXOPHYCEAE

Chroococcaceae

MICROCYSTIS FLOS-AQUAE (Wittr.) Kirchner *in* ENGLER-PRANTL, Natür. Pflanzenfam. 1(1a):56. 1900.

Cells 2–7 μ diam. Fig. 1.

Floating in pond near Brandsville, Howell Co., Aug. 21, 1928, *Drouet 176.*

Oscillatoriaceae

PHORMIDIUM CORIUM Gomont, Monogr. Oscill. 172. 1892.

Trichomes 3–5.6 μ diam. Type measures 3–4.5 μ diam. Sheaths violet when treated with chlor-zinc-iodine. Fig. 2.

"Stone-Lawrence Co. line," Sept. 10, 1898, *Wm. Trelease*, in Herb. Mo. Bot. Gard.

PHORMIDIUM PURPURASCENS (Kütz.) Gomont var. **elegans**, var. nov.

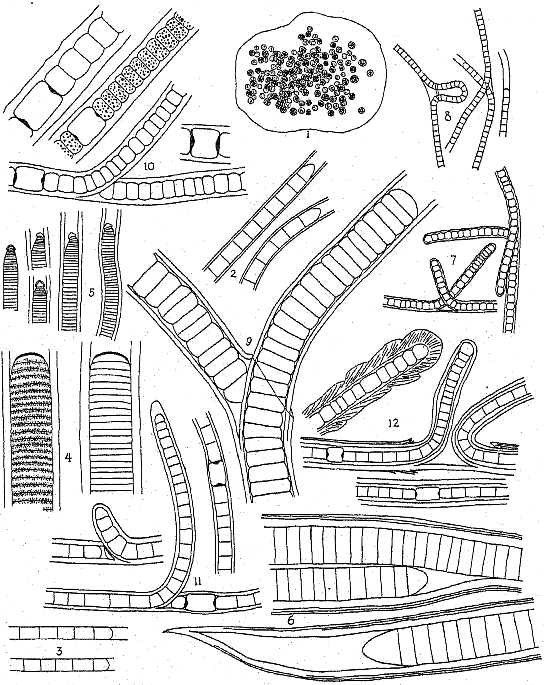
Var. cum trichomatibus 3–5.5 μ latis. Hab. in aqua artesia, Missouri.

Like the type, but with trichomes 3–3.5 μ diam. Fig. 3.

This alga formed violet-black leathery sheets on débris and water plants in a shady spring beside Highway 28 at the Gasconade River in Pulaski Co. The form answers exactly to the description of *Ph. purpurascens* (Kütz.) Gomont except for the larger size of the trichomes. The sheaths do not stain violet with chlor-zinc-iodine. Oct. 1, 1932, *Drouet 1030, 1033.*

LYNGBYA AESTUARII (Mertens) Liebm. forma *symplocoides* Gomont, *in* GEITLER, Cyanophyceae (Blaualgen). 1053. 1932.

Trichomes mostly 13–16.5 μ wide. Sheaths colorless with chlor-zinc-iodine. Fig. 4.



FIGS. 1–12.—Fig. 1, *Microcystis flos-aquae*, one colony showing gas vacuoles in cells. Fig. 2, *Phormidium corium*, ends of filaments. Fig. 3, *Ph. purpurascens* var. *elegans*, ends of filaments. Fig. 4, *Lyngbya aestuarii*. Fig. 5, *L. hahatonkensis*, showing variations of apices of trichomes. Fig. 6, *Hydrocoleus ravenellii*, showing typical end cell and presence of two trichomes within a sheath. Fig. 7, *Plectonema boryanum*, showing duplicate branching. Fig. 8, *P. notatum* var. *saegeri*. Fig. 9, *P. tomasinianum* var. *gracile*. Fig. 10, *Scytonema austinii*, showing variation in size of trichomes and habit of branching. Fig. 11, *S. hofmanni*. Fig. 12, *S. mirabile*. All ×600.

Forming *Symploca*-like mats on mud in a marsh fed by salt springs, Chouteau Springs, Cooper Co., May 3, Oct. 25, 1931, *Drouet 879, 933, 936*.

Lyngbya hahatonkensis sp. nov.

Strato nigrovirescente et laminoso; filamentis 6–7.5 μ diam., saepe plus minus curvatis et intricatis; vaginis chlorozincico iodurato non caerulescentibus, hyalinis, firmis, 1–2.5 μ latis; trichomatibus caeruleis, non constrictis, auguste attenuatis et ad apices vix curvatis, distincte capitatis; cellulis 4–5.2 μ crassis, brevissimis, 0.5–1.5 μ longis; cellula apicale conica, calyptram portante. Hab. in aqua artesia, Hahatonka, Missouri.

Filaments aggregated into dark blue-green strata, 6–7.5 μ in diameter, rather long, usually curved and entangled; sheaths well defined, not colored violet with chlor-zinc-iodine, hyaline, not laminated, sometimes roughened in age, 1–2.5 μ wide; trichomes bright blue-green (in living material), not constricted at cross walls, evidently narrowed at ends and slightly bent or spiraled, usually distinctly capitate; cells 4–5.2 μ in diameter, very short, 0.5–1.5 μ long; end cell conical, with conspicuous calyptra. Fig. 5.

In the drip of an artesian spring at Hahatonka, Camden Co., Aug. 9, 1928, *Drouet 148*.

The walls are usually somewhat granulated, although this is not always the case. Many granules appear throughout the protoplasm after the trichomes have been immersed in aqueous formalin. The form suggests *L. martensiana* var. *minor* Gardner, but in *L. hahatonkensis* the cells are much shorter and the ends of the trichomes more distinctly capitate (and calyptrate) and narrower. The latter objections also apply to *L. nigra* Ag. and *L. semiplena* J. Ag., to which it shows closest relationship.

HYDROCOLEUS RAVENELLII Wolle, Bull. Torr. Bot. Club **6**:183. 1877.

Trichomes 10–16 μ wide, on the average "ca. 12 μ." Sheaths not staining blue or violet with chlor-zinc-iodine, up to 8 μ wide. Fig. 6.

WOLLE's typical station is "pasture grounds, Houston, Texas"; our Missouri specimens develop in thick red crusts on soil after rainy seasons. GEITLER (4) cites the alga as growing "in stehenden Wasser."

Previously reported (2) from Boone Co., where it had been found on moist soil in the Perche Creek bottoms near Midway. Sept. 28, 1930, *Drouet 690*. Lately picked up "in an oak glade, soil dry and acid," near Pacific, Franklin Co., Nov. 20, 1932, *Ward Sharp*. Also on soil in a low meadow by Hinkson Creek south of Columbia, Boone Co., Apr. 8, 1933, *Drouet 1080*.

Scytonemataceae

PLECTONEMA BORYANUM Gomont, Bull. Soc. Bot. Fr. **46**:36. 1899.

Trichomes 1.3–2 μ diam. Sheaths not staining blue or violet with chlor-zinc-iodine. Fig. 7.

Appearing in the bottom of a culture dish in the laboratory at Columbia, Sept. 26, 1928, *Drouet 218*. To this species may be referred also a specimen marked "*Plectonema! boryanum?* Gomont. On pots, greenhouse," Missouri Botanical Garden, St. Louis, Mar. 24, 1918, *F. S. Collins 24*, in Herb. Mo. Bot. Gard. The material is not in an excellent state of preservation, so that I have not been able to make out the branching of the filaments. The habit of the trichomes otherwise appears to correspond with that of my material.

PLECTONEMA NOTATUM Schmidle var. **saegeri,** var. nov.

Var. cum trichomatibus 1.5–2.0 μ latis, indistincte articulatis. Hab. in aquariis, Columbia, Missouri.

Plant mass pale green or blue-green, laminated, floating or subaquatic; filaments parallel or entangled, long; false branching seldom, mostly in pairs; trichomes 1.5–2.0 μ wide, not constricted, with indistinct cross walls; cells half as long as wide to quadrate, seldom longer; cell contents pale blue-green, homogeneous; sheaths conspicuous, thin, colorless, not staining blue with chlor-zinc-iodine. Fig. 8.

In dilute Knop's solution with species of Lemnaceae grown by Dr. A. C. SAEGER, Columbia, Dec. 30, 1932, *Drouet 1050, 1051*. The filaments were especially abundant and well developed in the solution in which salts of manganese were absent and were less abundant in the solution containing such salts. In 3 per cent aqueous formalin (40% formaldehyde) the trichomes exhibit clearly the length of the cells, for by this treatment the walls become conspicuous and small granules are produced within the protoplasm.

PLECTONEMA TOMASINIANUM var. GRACILE Hansg. *in* GEITLER, Cyanophyceae (Blaualgen), 689. 1932.

Trichomes 12–13 μ diam. Sheaths staining blue with chlor-zinc-iodine. Fig. 9.

Reported previously (3). In Meramec River at Mincke, Franklin Co., and in the large lake at Lake Hill, St. Louis Co., Aug. 17 and 24, 1929, *Frances Holtzwart*. On mosses and rocks, Maramec Spring, Phelps Co , Nov. 1, 1931, and Mar. 21, 1932, *Drouet 950, 973*.

SCYTONEMA AUSTINII Wood, Smiths. Contrib. Knowl. **19**:58. 1872.

Trichomes 4–10 μ diam. Sheaths not staining blue or violet with chlor-zinc-iodine. Fig. 10.

Bluffs of the Osage River, Warsaw, Benton Co., Aug. 26, 1897, *Wm. Trelease*, in Herb. Mo. Bot. Gard.

SCYTONEMA HOFMANNI Agardh, *in* GEITLER, Cyanophyceae (Blaualgen). 772. 1932.

Trichomes 5–6 μ wide. Sheaths not staining blue or violet with chlor-zinc-iodine. Fig. 11.

"Big Spring, Current River, Carter Co." Likely on damp cliffs, Sept. 10, 1897, *Wm. Trelease*, in Herb. Mo. Bot. Gard. Reported by DR. HENRI HUS (5) and by MOORE and KARRER (6) from the Missouri Botanical Garden, St. Louis.

SCYTONEMA MIRABILE (Dillw.) Bornet, *in* GEITLER, Cyanophyceae (Blaualgen), 775. 1932. *S. figuratum* Ag.

Filaments 15–21 μ diam., trichomes (4–)6–10 μ diam. Sheaths not staining blue or violet with chlor-zinc-iodine. Fig. 12.

Cliffs at Big Spring, Carter Co., Sept. 10, 1897, *Wm. Trelease*, in Herb. Mo. Bot. Gard. Reported previously (2) from Montgomery Co., on a cliff at Bigsprings, Nov. 9, 1930, *Drouet 726*.

I wish to acknowledge the courteous cooperation of the members of the Department of Botany of the University of Missouri and of Dr. J. M. GREENMAN of the Missouri Botanical Garden.

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X-RAY DOSAGE IN RELATION TO GERMINATION OF PECAN NUTS

H. P. TRAUB AND H. J. MULLER[1]

(WITH ONE FIGURE)

As a part of the project in pecan breeding, the attempt is being made to induce mutations by means of short wave length radiation (2). Such a procedure appears justified since it has recently been shown that not all mutations induced by this means in living organisms are losses (4). When these experiments were undertaken, no workable technique for the control of pollination in the pecan had been reported (6). This made it necessary to postpone work on the irradiation of pecan pollen prior to its use in breeding experiments.

Results by various workers have shown that the number of mutations produced by short wave irradiation is a function of the total energy of ionization involved, that is, the number of electrons released and the speed and distance they travel, regardless of the source of the electrons (1, 3, 5). The initial experiments here reported are therefore logically concerned with the irradiation of pecan nuts (embryos) prior to planting, as a first step in determining the maximum possible x-ray dosage with still a fair percentage of germination.

Two varieties were employed in the experiment, Halbert and Payne. The particular lots included are described in table I. The Halbert lots were grown in three different localities.

The treatments were given with Victor Company broad-focus Coolidge tube with tungsten target and Snook transformer, at 50 kilovolts peak and 10 milliamperes, through a screen of aluminum 1 mm. thick, at a distance of 14 cm. from the target. The length of treatment ranged from none (check) to 160 minutes, beginning with 10 minutes and proceeding by geometrical progression to 160 minutes.

[1] The senior writer is now engaged in subtropical fruit production research and Dr. C. L. SMITH is continuing, in cooperation with Dr. H. J. MULLER, the work reported in this paper.

On April 16, 1931, the treated nuts and checks were treated in a dilute solution of $CuSO_4$ and then stored at 32°–34° F. in an electric refrigerator until May 4, when the nuts were stratified in sand and allowed to sprout in a cold frame. The results are presented in table II.

TABLE I

DESCRIPTION OF FOUR LOTS OF PECAN NUTS USED IN THE EXPERIMENT; CROP OF 1930

Variety	Where grown	Total weight (gm.)	No. of nuts per lb.	Percentage kernel	Shell thickness: mm.	Shell thickness: Percentage difference over Payne	Remarks
		Shell thickness 0.87–0.95 mm.					
Halbert no. 43	Southwest Texas	5.8	77	56	0.87	17	Grown on bottom land
Halbert no. 2	Central Oklahoma	5.8	78	57	0.87	17	Grown on bottom land
Halbert no. 170	Northwest Texas	6.5	69	54	0.95	10	Land naturally sub-irrigated
		Shell thickness 1.06 mm.					
Payne no. 183	Arizona	5.9	76	54	1.06	......	Land irrigated

The three lots of Halbert nuts showed marked variation in germinating power, 50, 70, and 100 per cent, as indicated by the check treatments (table II). In the case of the Payne nuts, the germination for the 20 minute treatment was as high (80%) as the check treatment, and the results for the 10 minute treatment were lower (65%) than the check. Germinating power apparently has not been accurately established on the basis of the small samples used. There appears to be a consistent decrease in percentage germination, however, due to increase in dosage after the 20 minute treatment in all cases: 70% to 0%, 37% to 0%, 40% to 0%, and 80% to 3% as

shown in table II. No Halbert nuts sprouted at 80 minutes' exposure; 23% Payne nuts germinated after treatment for 80 minutes, and 3% of the same variety after 160 minutes' treatment.

TABLE II

EFFECT OF VARIOUS DEGREES OF EXPOSURE TO X-RAYS ON GERMINATION OF PECAN NUTS; 1931

VARIETY	LENGTH OF TREATMENT (MIN.)	NUMBER OF NUTS	NUMBER GERMINATED ON						PERCENTAGE GERMINATED 8/27	REMARKS
			5/20	5/27	6/10	6/27	7/11	8/27		
			Shell thickness 0.87–0.95 mm.							
Halbert no. 43	None	10	1	2	5	5	5	7	70	1 died
	10	10	......	......	3	4	4	7	70	2 died
	20	14	......	......	5	5	5	6	42	......
	40	16	......	......	2	3	4	5	31	......
	80	10	......	......	......	......	......	......	0	......
	160	10	......	......	......	......	......	......	0	......
Halbert no. 2	None	16	......	......	1	6	6	8	50	1 died
	10	16	......	......	1	5	5	6	37	1 died
	20	16	......	......	......	......	......	6	37	......
	40	12	......	......	......	......	......	......	0	......
	80	16	......	......	......	......	......	......	0	......
	160	14	......	......	......	......	......	......	0	......
Halbert no. 170	None	10	1	3	9	10	10	10	100	2 died
	10	11	......	......	3	4	5	6	54	......
	20	10	......	......	3	3	3	4	40	1 died
	40	11	......	......	1	1	1	4	36	1 died
	80	11	......	......	......	......	......	......	0	......
	160	11	......	......	......	......	......	......	0	......
			Shell thickness 1.06 mm.							
Payne no. 183	None	30	......	......	10	20	23	24	80	......
	10	32	......	1	9	12	13	21	65	......
	20	31	......	2	7	18	20	25	80	......
	40	30	......	......	4	6	11	12	40	......
	80	30	......	......	2	2	6	7	23	1 died
	160	30	......	......	......	......	......	1	3	......

The plants in the case of the higher exposures were slower in sprouting, as shown in table II and figure 1. Later some of these late arrivals caught up, and in some cases even surpassed the checks.

Some of the embryos from treated nuts showed marked multiple sprouting and some plants remained dwarf and weak. It is of interest to note that, in many cases of longer exposure, the seed germ was killed but the cotyledons were partially or not at all damaged and functioned as living tissue. In a number of cases the tap root developed normally but no above-ground sprouts appeared (fig. 1.)

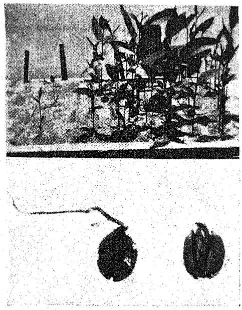

FIG. 1.—Upper: Payne seedlings, June 24, showing delayed germination for higher treatment. Lower: individual with root development only (on left); individual with seed germ destroyed (black) but cotyledons are growing and bursting nut (on right).

On the basis of these preliminary results, it appears that the maximum dosage with a reasonable percentage of germination, 20%, probably lies somewhere between 40 and 80 minutes' exposure under the conditions of the experiment.

It is shown in table I that the difference in shell thickness of Halbert lots as compared with the Payne lot ranged from 10 to 17% less for the former lots. In view of the relatively small numbers used, however, it would be hazardous to attempt to explain the difference in results on the basis of shell thickness. In future experiments the effect of this factor should be given some attention.

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CURRENT LITERATURE

The prairie

This comprehensive study is the third in a series of investigations on the prairies by WEAVER and his collaborators, HIMMEL and FITZPATRICK.[1] The previous works[2] dealt with the environment and the ecology of the dominant grasses. The present paper is concerned with the structure and dynamics of the vegetation and the ecology of the secondary grasses and forbs. The three constitute a very complete survey of the field.

The plan for the survey was to cover a representative area of large size. The region investigated measures about 300 miles north-south and 200 miles east-west; it includes portions of Kansas, Nebraska, Missouri, Iowa, South Dakota, and Minnesota. This is the heart of the "tall-grass prairie." One hundred thirty-five representative areas were studied, 20–360 acres in extent, distributed as uniformly as possible over the region.

Six principal types are recognized:

Upland types—

- Big bluestem consociation (*Andropogon furcatus*)
- Slough grass consocies (*Spartina michauxiana*)
- Tall panic grass-wild rye consocies (*Panicum virgatum*, *Elymus canadensis*)

Lowland types—

- Little bluestem consociation (*Andropogon scoparius*)
- Needle grass consociation (*Stipa spartea*)
- Prairie dropseed consociation (*Sporobolus heterolepis*)

These are first described as units. Detailed analysis follows, based upon very numerous quadrats. Distribution of major grasses within the consociations, analysis of the vegetation in the various types, relative height of grasses in different parts of the region are presented. In a previous paper (WEAVER and FITZPATRICK, *l.c.*) the ecology of the major grasses has been thoroughly treated; therefore, in this, particular emphasis is laid upon the minor grasses and especially the forbs. Upland and lowland species are considered separately, and under each of these headings there is further subdivision into a small group of important

[1] WEAVER, J. E., and FITZPATRICK, T. J., The prairie. Ecological Monographs 4: 109–295. 1934.

[2] WEAVER, J. E., and HIMMEL, W. J., The environment of the prairie. Cons. and Surv. Div. Univ. Nebr. Bull. 5. 1–50. 1931.

WEAVER, J. E., and FITZPATRICK, T. J., Ecology and relative importance of the dominants of tall-grass prairie. BOT. GAZ. 93: 113–150. 1932.

species and a larger one of those of lesser import. For each species there is very complete discussion of habit, ecological characteristics of root and shoot, life history, seasonal régime. The detailed study of individual species is followed by discussion of seasonal aspects, height growth and physiological activity, contacts with other major vegetation types, and invasion and succession.

In style, the work shows a happy combination of the scientific and the popular mode of presentation. It is pleasant reading and at the same time nothing of accuracy is lost. The aesthetic aspects of the prairie receive their full share of recognition. A point particularly worthy of commendation is the following through of complete seasonal development, both with respect to individuals and to communities. Even the winter aspect is not neglected. This has very seldom been adequately done for any vegetation type; it is possible only for a student living in intimate contact with the vegetation concerned year after year.

The paper is abundantly illustrated with excellent photographs, including typical views of communities and portraits of individual plants. In many cases, forbs were lifted with masses of accompanying grasses and photographed in the laboratory. This method gives an excellent individual portrait and at the same time shows the relation of the forb to the surrounding grasses.

We are indeed fortunate in having so complete and trustworthy a survey of this great vegetation type, which in its natural state seems doomed to inevitable extinction.—WM. S. COOPER.

A chapter of botanical history

The life of an outstanding scientist is not only interesting as a personal biography but his activities and contacts with fellow workers give a cross-section through a period of his science. This is especially true when the life is long and full of accomplishments. Such is the case with Professor GOTTLIEB HABERLANDT, whose memoirs[3] not only show the development of physiological plant anatomy but also give a survey of botanical history from NÄGELI, SCHWENDENER, DE BARY, SACHS, STRASBURGER, PFEFFER, and WIESNER to our day. There are primarily three great contributions which HABERLANDT gave to botany besides many minor ones: his handbook of physiological plant anatomy; his studies of the sense organs of plants; and his ecologic, physiologic, and organographic treatment of the tropical vegetation, a result of his visit to the botanical garden at Buitenzorg in Java. During his span of life, which will reach eighty years by November 28, 1934, HABERLANDT has seen the development of plant physiology as an independent branch in teaching botany. He himself organized an "Institut" for plant physiology and anatomy in Graz, Austria, and another one in Berlin. These institutes have their independent greenhouses and gardens, in spite of the existence of other large botanical gardens and buildings in both places.—A. C. NOÉ.

[3] HABERLANDT, G., Bekenntnisse und Beobachtungen. pp. 243. Julius Springer, Berlin. 1933.

GENERAL INDEX

Classified entries will be found under Contributors and Reviews. New names and names of new genera and species are printed in **bold-face** type; synonyms in *italics*.

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